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SEMIPERMEABILITY OF SEED COATS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 176

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(WITH NINE FIGURES)

I. Introduction

As our knowledge of the physical and chemical characteristics of seed coats increases, the importance of the physiological rôle and the biological significance of these structures become more and more apparent. Much work has been done upon the germination of seeds without an adequate knowledge of the real conditions offered to the embryos for their development. Many chemical substances and various ethereal stimuli have been used to influence the germination of seeds, and as a rule the seeds have been used with coats intact. This has been true especially of the Germans who belong to the vitalistic school. The assumption has been that the coats of seeds, and cell walls generally, are permeable to practically all water-soluble substances, and that the dead membranes and cell walls do not modify ethereal and chemical stimuli which act through them, simply because they are dead.

Recently a number of papers have appeared which have a very important bearing upon these problems. In 1907 BROWN (**10**) reported the discovery of a semipermeable membrane forming the outer layer of the seeds of *Hordeum vulgare* var. *coerulescens*, and later (**11**) published an account of the selective permeability of this outer dead membrane of the seed. This work was followed by

SCHRÖDER (**33**), who found the same kind of selectively permeable coat in wheat. ATKINS (**5**) failed to find this character in the coats of beans. He found that the absorption of water from living and dead seeds was identical until germination commenced, at which time the osmotic phenomena of the living cells were manifested. The forces concerned in the initial stages of water intake are, according to ATKINS, those of capillarity and imbibition; but on germination, osmotic pressure begins to influence the amount of water taken up by the living seeds. As SCHRÖDER has pointed out (**33**, p. 188, footnote), ATKINS failed to take into account the open micropyle of the Leguminosae used; but the same seeds on moist sand, with the micropyle turned up, absorbed 90 per cent of their dry weight from 10 per cent NaCl in 6 days, according to SCHRÖDER. And since the present paper was written, TJEBBES (**35**) has found that the seeds of the sugar beet probably have a selectively semipermeable membrane as part of the inner seed coat.

Up to the present, therefore, semipermeable membranes have been reported only in the Gramineae and Chenopodiaceae. However, another interesting discovery was made in 1907 by BECQUEREL (**7**), who showed that the thoroughly dried seed coats of certain plants were impervious to various gases and to such penetrating substances as absolute alcohol, chloroform, and ether. He made no attempt to determine whether these coats were also semipermeable.

During the last two years I have been investigating the character of the seed coat of *Xanthium* with special reference to the work of BECQUEREL, BROWN, and SCHRÖDER, and present here the results of the work. I wish to acknowledge with thanks the encouragement and helpful advice of Dr. WILLIAM CROCKER, and to express my appreciation of the excellent facilities afforded me by the Hull Botanical Laboratory.

II. Experimentation

1. MOISTURE AND PERMEABILITY

The discovery by BECQUEREL that various gases, and alcohol, chloroform, and ether would not penetrate certain seed coats if completely dried, seemed so unusual that attempts were made to repeat his experiments, using the testa of *Xanthium glabratum*

cemented over the end of a glass tube fitted with a perforated rubber cork, the tube being then filled with mercury, and set up as a sort of barometer. Although the coats were supported by strong cloth, they were too delicate to withstand the strain, and would always burst on being inverted over the mercury cup. Shorter columns also were tried. In one case a column of mercury 20 cm. in height was sustained for four days without any diffusion of atmospheric gas taking place through the membrane.

Several attempts were made to test the diffusion of oxygen through the dry seed coat by means of an apparatus like that used by CROCKER (**14**) to test the diffusion through moist coats. These tests ran for only a few hours at a time and the results were negative. No measurable diffusion was noted, but the slightest injury to the coat was found to permit a rapid passage of oxygen.

Much better results were obtained with the experiments on the permeability of dry coats to ether, chloroform, acetone, and absolute alcohol. Seeds of *Xanthium glabratum* were dried for two weeks at 40° C., then put into a desiccator over phosphoric anhydride at 10 mm. atmospheric pressure for 18 days at 40° C., after which they were stored in a similar desiccator for use.

Water-free ether was prepared by treating the best ether obtainable with alcohol-washed KOH in sticks for 12 hours, and then distilling it with ribboned metallic sodium in both distilling and receiving flask. The sodium was allowed to act in the receiving flask until every trace of water had disappeared.

Five lots of dry seeds were then immersed in dry ether, and kept at a constant temperature in a Freas thermostat for various lengths of time from 2 to 36 days. On removing them from the ether the seeds were carefully dried, soaked in water, the testas removed, and the embryos placed in germinative conditions; 87 per cent of the seeds germinated, and the young plants were just as vigorous as the untreated controls. In those sets of seeds soaked for 16 and 36 days respectively, 100 per cent of both uppers and lowers germinated, and the average growth in length in 6 days was for the uppers 9.5 cm., for the lowers 11 cm.

Seeds carefully dried were also placed in chloroform, absolute alcohol, and acetone without the precaution of drying the fluids.

In all cases the dried seeds remained for many days (16–30) in the fluids without showing any injury, as shown by their normal germination afterward. That these fluids do not penetrate the testa can be demonstrated indirectly by using seeds with defective coats. In no instance were such seeds found to be viable after more than about 15 hours' immersion in absolute alcohol, ether, chloroform, or acetone. The length of time seeds with broken coats can remain alive in these substances depends upon the size and position of the defect. If the break occurs immediately over the hypocotyl, a few hours' immersion will kill the seeds; but if at the tips of the cotyledons, they will remain viable some hours longer.

The question naturally arises as to how much moisture can be put into these liquids, and still leave the seeds uninjured by long soaking. Commercial 95 per cent alcohol was found not to kill merely air-dried seeds in 4 days' time, but had killed them within 8 days. A series was then run in 90, 80, 70, 50, and 35 per cent alcohol. In a few hours the seeds in the lowest three grades had become excessively swollen and semitranslucent; they were found to be dead. Those in 80 per cent were by no means so swollen, but had been killed. Those in 90 per cent were apparently hard and sound, but at the end of 3 days all were dead. They may have been killed in considerably shorter time than this, as they were not tested for germination till the end of 3 days.

The results of these experiments confirm the findings of BECQUEREL, who kept various seeds in alcohol, ether, and chloroform for a full year without any serious loss of viability. While these facts seem remarkable, they merely confirm the results reported a good many years ago by GIGLIOLI (**21**), who obtained, for instance, the germination of 20 per cent of alfalfa seeds after more than 16 years in a saturated solution of $HgCl_2$ in absolute alcohol. GIGLIOLI says that many of these seeds produced plants which flowered and fruited normally after this long exposure to destructive gases and liquids. The protective nature of the dry seed coats of certain seeds, therefore, is fully established, and is due to impermeability.

The results of these experiments offer no evidence against BECQUEREL's view that gases, especially oxygen, are not able to

diffuse through the thoroughly dried seed coats of certain seeds. CROCKER (**14**) was inclined to believe, from his study of the respiratory ratio in *Xanthium* seeds, that more oxygen diffused through the dry seed coats than through those which had not been thoroughly dried. This conclusion is probably shown now to be incorrect. The gas exchange noted by him in the case of dried coats may have occurred at least partially through defects in the coats; for, as I shall show later, there are a good many seeds which prove to have defects which are invisible, even on microscopic examination, and these defects may possibly be increased in number by the process of drying.

2. SELECTIVE SEMIPERMEABILITY OF XANTHIUM SEED COATS

A few preliminary tests to determine the amount and rate of imbibition of *Xanthium* seeds in solutions of sodium chloride of various strengths indicated the existence of a very efficient semipermeable membrane in the seed coat. Water enters the seed very rapidly under the powerful forces of capillarity and imbibition until more than 50 per cent of the air-dry weight of the seeds has been taken up. Nearly all of the water is taken up in 12–15 hours, as shown by the curves in figs. 1 and 2.

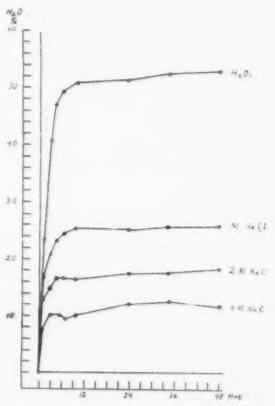

FIG. 1.—Curves of imbibition for seeds of *Xanthium* in salt solutions of various strengths, and in water; time element plotted on the axis of abscissae, percentage increase over air-dry weight on the axis of ordinates.

This rapid intake of water is in sharp contrast with that which occurs in the Gramineae. In the seeds of the latter the rate of water intake is considerably slower, and extends over 7 or 8 days or even longer, before saturation is reached. Evidently the protective structures influence the rate at which water passes into the seeds.

When strong salt solutions instead of distilled water are used with *Xanthium* seeds, the powerful internal forces, capillarity, imbibition, and perhaps the chemical

attraction of colloids, cause the water to enter with almost as great initial rapidity as when pure water is used, until the osmotic pressure of the salt on the outside balances the internal forces mentioned; then the entrance of water quickly ceases. After equilibrium is established in any given salt solution, increasing or decreasing the density of the solution extracts water from the seed or permits water to enter it in accordance with the direction of the disturbance. The adjustment continues rapidly after the disturbance until the equilibrium is reestablished, when the amount again remains constant.

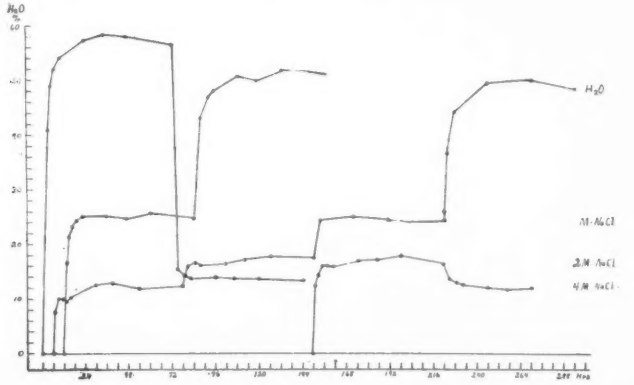

FIG. 2.—Curves showing entrance and withdrawal of water in *Xanthium* seeds on shifting from water to salt solutions, or vice versa, at three-day intervals; the semipermeability of the membrane is well illustrated by the behavior recorded in these curves; curves 2, 3, and 4 are displaced to the right to avoid confusion.

The speed with which this adjustment of internal and external forces takes place in *Xanthium* is again in contrast with the rate of adjustment in grasses, such as wheat and barley, in which the rate of adjustment is slow, requiring days rather than hours to establish equilibrium. It appears that the coat in *Xanthium* offers very slight resistance to the passage of water, while in the Gramineae a comparatively much greater resistance is offered by the coat structures.

This relation of osmotic pressure to capillary and imbibition forces, chemical and physical, through the semipermeable coat of the *Xanthium* seed, is splendidly illustrated by figs. 1 and 2, which

show the imbibition curves for distilled water, and for molecular, two-molecular, and four-molecular solutions of NaCl as the seeds were shifted from one strength to another at three-day intervals. The close agreement of the constant portion of the curves in each strength of solution should be noted. The character of the curve during the last two days of each three-day interval indicates that the salt does not enter in appreciable amounts. Comparison with the type of curve given by SCHRÖDER (**33,** p. 189) for wheat shows that this membrane is at least as efficient in excluding NaCl as the coats of the Gramineae.

By appropriate chemical methods it has been shown that no passage of the salt through the membrane occurs. It was necessary, however, to test seeds singly, since one finds rather frequently seeds which have invisible defects in the coat which allow the passage of salts.

Many other chemical substances, usually in molecular solutions, were used to determine the range of selective semipermeability. The permeability or non-permeability to each substance was judged by the amount of imbibition water taken up from such solutions of acids, bases, and salts. Anyone who investigates the subject will be convinced that this means of determining semipermeability is sufficiently accurate for all ordinary purposes. The results, in percentage of increase over air-dry weight by imbibition, are given in table I.

From this table it is seen that the nitrates as a class, and especially silver nitrate, penetrate the coats. Iron sulphate slowly enters, while copper sulphate does not. The penetration of copper sulphate can be detected, if it occurs, by removal of the testa and examination of the embryo. Occasionally as high as 20–40 per cent of the seeds show slight localized penetration of $CuSO_4$ through defects in the coat too slight to be seen on microscopic examination of the dry seeds. Because of these defects the figures given for any salt are approximations only; but in case there is general permeability it is easily recognized by the amount of imbibition. Since 60–80 per cent of the seeds show no penetration even after prolonged soaking in $CuSO_4$, and since they retain their vitality perfectly, notwithstanding the very highly poisonous character of

the solution, it seems reasonably certain that copper sulphate does not penetrate a sound testa.

TABLE I

PERCENTAGE OF IMBIBITION

(BASED ON AIR-DRY WEIGHT)

OSMOTIC PRESSURE (CALCULATED FROM THE LANDOLT-BÖRNSTEIN TABLES)* IN ATMOSPHERES	SUBSTANCE	END OF THREE DAYS		TEN DAYS	REMARKS
		Exp. I	Exp. II	Exp. II	
	Water	54.94	53.95	.....	
38.02.....	M–NaCl	26.	24.46	.....	
72.01.....	2M–NaCl	21.58	18.47	.....	
130.62.....	4M–NaCl	12.16	13.44	.....	
	Sat. NaCl	7.12	6.2	.....	End of two days
35.53.....	M–$AgNO_3$	49.83	46.57	70.89	
37.66.....	M–NH_4NO_3	32.47	33.	40.6	
37.67.....	M–$NaNO_3$	34.18	28.88	32.09	
36.53.....	M–KNO_3	37.89	38.87	41.29	
28.25.....	M–$CuSO_4$	34.48	35.4	35.59	
	M–$FeSO_4$	34.39	37.48	44.73	
39.6.....	M–KCl	35.15	37.62	38.3	
	M–K_2CrO_4	24.55	32.04	36.91	
	M–$Na_2S_2O_3$	28.37	.....	.....	
	5% $HgCl_2$	70.2	.....	.....	
	CH_3OH	66.6	.....	.....	10 volume per cent
	C_2H_5OH	57.14	.....	.....	" " " "
	C_3H_7OH	69.25	.....	.....	" " " "
	Glycerol	29.32	.....	.....	" " " "
	Ether sat.	53.95	.....	.....	End of 5 hrs.
	Iodine, 5 per cent in KI	.....	.....	.....	Penetrates
	M–Sucrose	34.41	.....	.....	
	M–Fructose	35.54	.....	.....	
	M–Glucose	36.51	.....	.....	
	M–Lactose	35.47	.....	.....	
	M–KOH	79.	.....	.....	In 23 hrs.
	M/10 KOH	72.2	.....	.....	
	M–NaOH	102.06	.....	.....	In 35 hrs.
	M/10 NaOH	50.2	.....	.....	In 10 hrs.
	M–HCl	32.87	.....	.....	
	M–H_2SO_4	42.06	.....	.....	
	M–HNO_3	73.33	.....	.....	In 48 hrs.
	M–Tartaric	36.87	.....	.....	
	M–Acetic	57.3	.....	.....	In 11 hrs.
	M–Lactic	55.84	.....	.....	
	M–Citric	37.06	.....	.....	

*In these calculations from the Landolt-Börnstein tables correction is made only for dissociation. The osmotic pressure of 2M and 4M—NaCl is probably somewhat higher than here indicated, due to the formation of hydration compounds. Saturated NaCl is believed to exert 375 atmospheres of osmotic pressure.

Mercury bichloride enters the seed rapidly, as do also aqueous solutions of iodine in KI, the monatomic alcohols, ether, the alkalies,

and most of the acids, except perhaps hydrochloric and tartaric. The entrance of sulphuric is very slow. The triatomic alcohol, glycerol, does not enter, nor do the sugars.

The semipermeability is not dependent on any living cells in the membrane, but is purely a physical phenomenon. Boiling the seeds does not destroy this character of the coat, nor do such poisons as $HgCl_2$, $AgNO_3$, iodine, the alcohols, ether, etc. Solutions of sodium chloride extract water rapidly from seeds which have imbibed almost 70 per cent of their weight in 5 per cent $HgCl_2$. The peculiarity of the testa must therefore depend upon the physical structure and chemical composition of the dead cell walls, and upon the relations it may assume toward the various solutes and solvents, not upon any vital conditions.

The seed coat of *Xanthium* possesses a great advantage over the membranes discovered by BROWN and SCHRÖDER in barley and wheat as an object of research; for in *Xanthium* the entire coat is easily removed after soaking for a few hours in water, and can then be used readily as an osmotic membrane.

FIG. 3.—Osmotic apparatus used in testing directly the semipermeability of *Xanthium* seed coats; description in text.

For this purpose I have used the apparatus illustrated in fig. 3. A glass tube about 6 cm. in length and 1.5 cm. in diameter is closed at both ends with short rubber corks, each perforated by a hole 3 mm. in diameter. Since the corks must fit tightly into the glass tube, the perforation of one of the corks is prevented from collapsing by inserting a short piece of glass tubing 3 mm. in diameter. The testa is prepared for use by cutting it longitudinally along one side, and cutting off the ends. The testa can then be opened out and dried under a weight, thus producing a rectangular membrane about 6×8 mm.

This membrane is cemented carefully to the rubber cork which has been prevented from collapsing by the narrow glass tubing. It is best to place a thin layer of wax over the cork first, then press the membrane firmly into the wax, after which the edges of the dry

membrane are carefully sealed with more of the wax, leaving only that portion of the membrane immediately over the hole in the cork entirely free from wax. In my experiments the area of membrane left uncovered has been approximately equal to the area of the perforation through the cork.

The 6 cm. glass tube is now placed in vertical position, with the membrane at the lower end. A 4 M solution of NaCl is introduced through the upper cork until the chamber is quite filled. Care must be taken that small bubbles of air which tend to remain in the perforation of the lower cork while the chamber is being filled do not prevent the solution from filling this perforation. Otherwise the membrane will not be in contact with both water and solution.

Finally a long glass tube 3 mm. in diameter is carefully inserted through the upper cork. The salt solution will rise a short distance in the narrow tube, due to displacement. After making sure that the apparatus is entirely free from salt solution externally, the chamber is suspended in a vessel of distilled water with the liquids at the same level, just as if it were a thistle tube. This apparatus and the experiment itself are so simple that they should be of considerable pedagogical interest, as demonstrating directly the independence of semipermeability of this kind from any vital matter.

In one such experiment a rise of 175 mm. occurred in the narrow tube in 7 days. This is a fairly rapid rise, inasmuch as the tube in which the rise was measured had practically the same cross-section area as the diffusion membrane. It means that on the average a column of water 25 mm. long passed through the membrane in a day's time. The rate of passage is so rapid as to indicate a very slight resistance of the membrane to the passage of water, a condition quite different from that found in the Gramineae, as already suggested.

Here again the impermeability of the membrane was demonstrated by dropping some of the liquid taken from immediately below the diffusion membrane into $AgNO_3$. No visible precipitate was obtained, even at the end of 7 days.

3. STRUCTURE AND COMPOSITION OF THE TESTA

The morphological development of the seed coat of *Xanthium* has not been fully traced, but it is probable that the seed coat is

formed in the same way as in another of the Compositae, *Helianthus*, the structure of which has been reported by BRANDZA (**9**).

In *Helianthus* the inner layer of the 3-layered seed coat is formed from the nucellar epidermis, and is composed of just one layer of cutinized cells. *Xanthium* likewise has a 3-layered testa, the inner layer of which is a single layer of cells excepting over the hypocotyl, where, as CROCKER has shown, it is several cells thick. It is probable that this inner layer, with the exception of its chalazal portion, which lies immediately over the hypocotyl, is derived from the epidermis of the nucellus.

At first it was suspected that the inner stratum might be the semipermeable layer, although CROCKER had suggested in 1906 that the thicker middle one probably excluded oxygen. While attempt was being made to locate the seat of the semipermeability it was discovered that certain substances, the higher alcohols, acetic acid, and the alkalies, occasionally cause a bursting of the middle layer without injuring the single-celled inner lamina. The outer layer, of course, is too loose and chaffy to function osmotically.

In the case of M and M/10 solutions of KOH and NaOH, the bursting of the middle layer is followed by a periclinal separation of the inner layer from the middle one, which is probably the ovular integument, so that the embryo, surrounded by the intact, thin, delicate, one-celled inner layer can be removed from the external coats. This discovery was a very important aid in studying the semipermeability of the different layers of the testa, and illustrates the advantage which removable coats have over such as have been found in the grains. In the barley and wheat all such investigations had to be indirect, because the membrane could not be removed or separated into distinct layers.

The thin inner coat was shown to be a very efficient semipermeable membrane by treating the much swollen seeds surrounded by this membrane with 4M–NaCl. At the time one such seed was introduced into the salt solution it weighed 110 mg. After one hour the membrane was completely collapsed and pressed very tightly against the embryo. It weighed 58 mg. In 4 hours it weighed 52 mg., in 10 hours 51 mg., after which a rise in weight gradually occurred, probably due to a very slow entrance of NaCl.

In 2 days its weight was 59 mg. Fig. 4 shows the curve for loss of water from such a seed which weighed 198.5 mg. at the time it was put into NaCl.

The middle layer, which is several cells thick, was subjected to a direct test by using it as an osmotic membrane cemented over a rubber cork. Its osmotic character is demonstrated by the following figures:

Rise	Time	Rise	Time
45 mm	5 hrs.	159 mm	32 hrs.
72 "	9 "	169 "	48 "
125 "	20 "	171 "	72 "

However, this layer is by no means as efficient as the inner layer. The NaCl could be seen streaming through the membrane into the distilled water, causing the same appearance as the rapid solution of a salt in water.

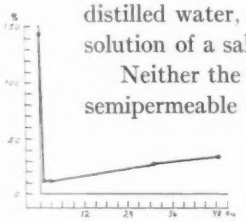

FIG. 4.—Curve showing loss of water by a seed which had become excessively swollen in M–NaOH when later put into 4M–NaCl; the outer coats of the testa had burst, leaving the inner coat, one cell thick, intact; the curve demonstrates the semipermeable character of the inner layer.

Neither the middle nor inner membrane is as perfectly semipermeable alone as are both together in the natural condition. Microchemical study of the coat, using NaCl and $AgNO_3$, had given evidence in the same direction, that the middle layer allowed salts to reach the inner layer, but that the precipitate of AgCl was not certainly present within the inner layer. The extreme stretching of the coats preceding the bursting, or a physical or chemical modification due to the macerating agent, may be responsible for the impaired efficiency of the separated membranes.

The cellular arrangement in the inner membrane is shown in fig. 5. Microchemical tests showed the walls of this layer to be largely cellulose, with little or no suberization. A dark blue color was obtained on treatment with sulphuric acid and iodine, and the materials of which it is composed are almost completely dissolved in Schweizer's reagent. Practically no coloration was obtained with alkanin and other suberin reagents, except in the cell contents, and these are known not to be respon-

sible for this physical character of the membrane, which remains unchanged when the cell contents have been removed by dissolution.

REICHARD (**29**) some years ago showed that the coats of *Hordeum* are full of tannin, and has now (**30**) raised the question whether the tannin present there has any effect on the semipermeability of its membranes. The colloidal condition of the tannin, and its known peculiarities chemically, suggested that it might be in a large measure responsible for the physical properties of the coat. He investigated the position of the tannin by microchemical methods, and found that in sections the barley grains showed a sharply defined layer of tannin which could easily form a continuous coat. Treatment of seeds with tannin solvents showed that with such treatment the layer of tannin becomes broader and more diffused. Alcohol (96 per cent) used alone does not dissolve tannin. Following the alcohol treatment with iron sulphate solution as a tannin stain shows that the tannin layer becomes if anything more sharply defined in alcohol of high grade. But if the seeds are soaked first in water, then the tannin shows marked solution effects when subsequently treated with alcohol. Alcohol seems to dissolve tannin only when it has been previously wet.

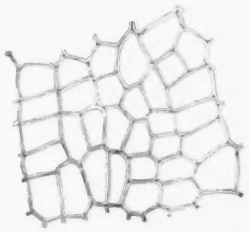

FIG. 5.—Cellular arrangement of a portion of the inner layer of the testa.—Camera lucida, 4 mm. obj., 4 ocular, 160 mm. tube.

According to REICHARD'S view, the entrance of substances into the seed would depend upon their ability to dissolve the tannin layer, or to dissolve in it. Since strong alcohol does not dissolve tannin, seeds with such a tannin layer might lie in absolute alcohol indefinitely without loss of vitality. But the presence of sufficient moisture to dissolve or partially dissolve the tannin would allow rapid entrance of alcohol, and a consequent killing of the embryo. The bearing of these facts upon the resistance of dry seeds to organic solvents discussed in an earlier section of this paper is obvious.

Hydrates, especially NaOH, have a powerful solvent effect upon tannin; even in M/10 solutions the solvent action is apparent, and

as the concentration increases, the action is very rapid. On the other hand, acids do not dissolve tannins, but rather precipitate them from solution, according to REICHARD.

The barley grains used by REICHARD did not seem to be as perfectly semipermeable as those used by BROWN. This variation in semipermeability is attributed to differences in the tannin content from differences in the ripening process. He believes that the tannin is an "assimilation" product of chlorophyll, and that in unripe seeds the tannin has not been fully deposited in the seed coats. Or one can assume that the tannin for some unknown reason has failed to reach the proper chemico-physical condition for complete semipermeability.

While it seems entirely possible that the resistance of dry seeds to alcohol and other reagents such as ether, chloroform, etc., might be related in certain cases to tannin in the seed coats, it does not seem probable to me that semipermeability of these membranes can be explained on these grounds.

There is a considerable amount of tannin in the testa of *Xanthium*, as shown by treatment with ferric chloride, ferric sulphate, potassium dichromate, etc. But the tannin in this case does not seem to form so definite a layer as REICHARD reports for *Hordeum*. It seems to be scattered through the tissue in minute granules, occurring especially in the outer and middle layers of the coat with only very minute quantities in the inner layer. As has been shown, treatment of the coats with molecular NaOH, which is a strong tannin solvent, according to REICHARD, does not destroy its semipermeable character. For these reasons it is the writer's opinion that the property belongs to the cellulose, rather than to substances associated with it in the coat. Further microchemical studies upon the seed coat of *Xanthium* are in progress, and in time it may be possible to analyze the conditions which cause the exclusion of certain salts and the passage of others through the semipermeable coat of seeds.

4. SEMIPERMEABILITY A WIDESPREAD PHENOMENON

In addition to the work on *Xanthium*, a number of seeds in various families have been tested. The results show that the

lifeless membranes forming the coats of seeds frequently exhibit osmotic effects. A number of widely separated families are represented in the list now known to possess such properties: Alismaceae (*Alisma Plantago-aquatica*), Gramineae (barley, wheat, oats, etc.; probably most grasses), Chenopodiaceae (sugar beet), Rosaceae (peach, apple), Leguminosae (*Vicia Faba*, scarlet runner, lima bean), Compositae (*Xanthium*, *Helianthus annuus*). It should be said at once that these seeds do not all exhibit the same degree of semipermeability. The coat of *Xanthium* apparently permits no passage or only very slow passage of certain solutes, comparing favorably with the coats of *Hordeum* and *Triticum* in this respect. Coats of the sunflower and peach are only less efficient, while the bean coats usually allow a noticeable passage of salts. The Leguminosae seem to be less uniform in their behavior than any seeds tested. Certain specimens have very good semipermeable membranes, others have rather poor ones.

As pointed out in the introduction, ATKINS found Leguminosae not to possess semipermeable testas, but had overlooked the open micropyles. Direct methods of testing proved that these membranes do act osmotically. It was not found practicable to wax these membranes onto rubber corks, because the great amount of expansion of the membrane on soaking, after the apparatus was arranged, always resulted in breaking the coat loose from the wax. To overcome this difficulty, the wet bean coats were placed tightly between two perforated rubber corks which were smeared slightly with vaseline, and used just as in the case of *Xanthium* already described. In this case the hole in the cork below the membrane must also be carefully filled with water to insure contact of both fluids with the membrane.

Coats of *Vicia Faba* used with saturated NaCl solution gave a rise of 72 mm. in 36 hours; and of scarlet runner a rise of 135 mm. in 10 days, the rise continuing throughout this time, but amounting to only 17 mm. during the last 4 days. The escape of the salt through the coats of both these legumes was readily demonstrated by use of $AgNO_3$.

It is a pleasure to record the fact that Professor STEVENS of the University of Kansas has for more than 8 years been using the coats

of the lima bean to demonstrate osmosis in his elementary classes in plant physiology. The coats are merely tied over the ends of glass tubes. His experience has been that the coats are irregular in their behavior, and that successful use demands the testing of the membranes, and the choice of those whose behavior with a definite strength of solution is uniform. Thus chosen, the membranes may be used to demonstrate admirably PFEFFER's discovery that osmotic pressure varies directly with the concentration of the solution.

Recently LAVISON (**24**) studied the entrance of salts into the roots of plants and showed that the cellulose "frames" (*cadres*) around the cells of the endodermis are impermeable to certain salts, and that entering salts must therefore pass through protoplasm in penetrating the root beyond the endodermis. Later (**25**) he made the further observation that the cellulose cell walls forming the periclinal walls of the endodermis behave toward entering salts like the protoplasm itself. That is, those salts which are excluded by the protoplasm are excluded by the walls also. LAVISON does not say that the walls are selectively semipermeable, but that is seemingly the only possible interpretation of his observation. It should be noted that this applies only to the *young* cellulose walls.

All of this evidence points to semipermeability as a widespread phenomenon among lifeless plant membranes. Of course, the membrane must be permeable to water if it is to be osmotically active. The thin skin of potatoes is impermeable to water solutions, allowing neither salt nor water to pass through. The possibly semipermeable character of cellulose membranes cannot be overlooked in future investigations dealing with the entrance of salts into plant tissues.

5. OSMOTIC PRESSURE AND IMBIBITION FORCES

The experiments upon *Xanthium* seeds with concentrated salt solutions suggested the possibility of measuring the capillary and imbibition forces of seeds by use of solutions of very high known osmotic pressure, since the balancing of osmotic pressure on the one hand against capillarity and imbibition on the other through a perfect semipermeable membrane is a very simple matter.

For this purpose saturated solutions of lithium chloride, which

gives the highest osmotic pressure of any known neutral salt, was employed. The imbibition curve for air-dry seeds known to contain 8–9 per cent of moisture is shown in fig. 6. In the case of both lower and upper seeds there was found a slight loss in weight during the first few hours of soaking in this concentrated LiCl solution. The loss is very small in amount, averaging 0.5 mg. per seed in all the tests made. But after the seeds have been in the solution for 6 or 7 hours, there is a very slow increase in weight, so that at the end of 46 hours the weight of the soaked seeds was the same as when first put into the fluid. The exact significance of this behavior is not very clear, but it is obvious that these seeds do not possess internal forces of sufficient magnitude to withdraw any water from the LiCl solution.

FIG. 6.—Imbibition curve for air-dry seeds in saturated LiCl solution.

In order to test the balance between osmotic pressure and the internal forces further, a number of seeds were soaked first in water until they had taken up about 44 per cent of their dry weight. They were then placed in saturated LiCl, which was kept saturated by keeping 40–50 grams of the undissolved salt in the bottom of the vessel and stirring it up frequently. The imbibition curve is shown in fig. 7. At the end of one hour, more than three-fourths of the water had been withdrawn, and in 7 hours the seeds were only 2.5 per cent above their air-dry weight. The water was withdrawn more and more slowly until at the end of 100 hours they reached their original dry weight. The results shown in figs. 6 and 7 indicate that capillarity and imbibition force in an air-dry seed of *Xanthium*, that is, one with 8–9 per cent of moisture, is approximately equal to the osmotic pressure of a saturated solution of LiCl.

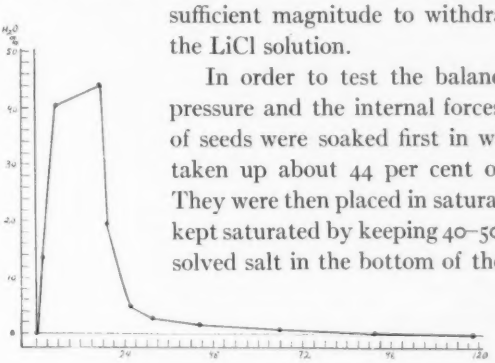

FIG. 7.—Curve showing loss of water from seeds soaked in water before transferring to saturated LiCl solution; imbibition force of air-dry seeds and osmotic pressure of saturated LiCl solution approximately equal.

One of the most difficult problems was to secure adequate data as to the size of the pressures and forces indicated by the results. The literature aside from the work of the physical chemists contains serious disagreements, and the physical chemists have done very little on the osmotic pressure of concentrated solutions. Moreover, there is wide divergence in the interpretation of the little they have done. As the writer is not a physical chemist he has been at a distinct disadvantage in the attempt to clarify a very confusing situation.

In his work on *Hordeum*, BROWN ascribes to a saturated solution of NaCl an osmotic pressure of 125 atmospheres. A few years earlier, RACIBORSKI (**28**), who investigated the upper limits of osmotic pressure in living plant cells, cites DIETERICI as authority for the statement that saturated NaCl has at 20° C. an osmotic pressure of 375 atmospheres. RACIBORSKI gives the pressure as 349.11 atmospheres at 0° C., the saturation concentration being 35.51 per cent. The figures given by BROWN are apparently based on the assumption that the law announced by VAN'T HOFF in 1887, that osmotic pressure is proportional to concentration, a law based on PFEFFER'S work a decade earlier, applies to all concentrations whatsoever, without any allowance for electrolytic dissociation or hydration, both of which must play an important rôle in the pressure of these concentrated solutions. On such an assumption the pressure should be near 125 atmospheres.

That this figure is too low can readily be determined from data as to the electrical conductivity of NaCl derived from the Landolt-Börnstein tables. A 4 M solution would exceed 130 atmospheres, so that a saturated solution should run to 160 atmospheres or beyond, merely due to ionization.

Moreover, there is reason for believing that this law of the relation of osmotic pressure to concentration holds only for lower concentrations, and that still further corrections are necessary in the case of highly concentrated solutions; for the actually observed pressures depart widely from the theoretical requirements as concentration increases. BERKELEY and HARTLEY (**8**) measured the osmotic pressure of solutions of sucrose, dextrose, and galactose by an igenious method, and have shown that dilute solutions give

pressures agreeing with the gas laws. But curves representing the pressures in higher concentrations show that the osmotic pressure increases in strong solutions much more rapidly than the molecular concentration.

Formerly it was customary to calculate the osmotic pressure on the basis of solution volumes. MORSE, FRASER, and others, however, found a better agreement between increase of concentration and osmotic pressure when the concentration is referred to a liter of solvent rather than to a liter of solution. RENNER (**31**) has recently given an excellent summary of the literature on the calculation of osmotic pressures, and holds that this change from liter of solution to liter of solvent will bring into agreement the pressures obtained by plasmolytic and those obtained by cryoscopic methods, the former having been in error.

Although making this change brings agreement between theoretical and observed pressures for more concentrated solutions than was true with the old method of making up molecular solutions, the observed pressures are still considerably above the pressures demanded by our theories, after all these corrections and improvements in method have been made.

The relation of the actual pressures found by BERKELEY and HARTLEY to the pressures demanded theoretically by both methods of calculating pressure from concentration is shown graphically by PHILIP (**27,** p. 53) by means of curves. RENNER also shows a similar diagram in his recent paper but does not mention PHILIP's discussion. There is little doubt of the correctness of the pressures found by the direct methods of measurement employed by BERKELEY and HARTLEY, as their results have been confirmed recently by TROUTON (**36**), who uses an entirely new method for the direct measurement. Ether will not take up as much water from a solution as it will from pure water. He determines the pressure necessary to force as much water from a solution of sucrose into ether as the ether will take up normally from pure water (1.05 per cent). The pressure required to force the water content of ether up to 1.05 per cent from a solution of sucrose containing 600 grams per liter was 80 atmospheres. BERKELEY's result was 81 atmospheres with the same strength of sucrose solution, an agreement well within the limits of error.

The findings of BERKELEY and HARTLEY were discussed by CALLENDAR (**13**), who offers a plausible explanation for the high pressures observed. He suggests that the solute probably enters into a molecular complex with the solvent, forming hydration compounds. This chemical combination of solute with solvent reduces the number of free molecules of water in the solvent, decreases therefore the vapor tension, or, what amounts to the same thing, increases the osmotic pressure; for, as CALLENDAR says: "There is a definite and simple relation between vapor pressure and osmotic pressure which has been verified by Lord BERKELEY and HARTLEY for strong solutions."

The degree of hydration is represented by a in CALLENDAR'S discussion, and he believes $a=5$ in the case of the sucrose solution used by BERKELEY; and in the case of KAHLENBERG'S (**23**) observations on the rise of boiling-points in concentrated solutions of NaCl, the curve agrees with a hydration value of $a=6$ up to a concentration in which there are 6 molecules of NaCl present for every 100 molecules of water (CALLENDAR **13**, p. 493, diagram p. 492).

CALLENDAR assumes that the molecular complex formed by the hydration is a *definite* chemical compound at any given concentration. In this he does not agree with JONES and BASSETT (**22**), who claim to have shown that such hydration compounds are indefinite, and may vary from a few to many molecules of water to each molecule of solute in any given concentrated solution.

If the compounds formed were indefinite, it would not be possible to explain the increased osmotic pressure of strong solutions by hydration; but CALLENDAR points out that the determinations of JONES and his co-workers are erroneous at various places, and their conclusions untrustworthy. The writer can only express it as his opinion that CALLENDAR'S assumption is probably the correct one, that the hydration compounds are definite, and that the osmotic pressure of concentrated solutions depends partly upon the hydration value of the molecular complex formed.

If this opinion is correct, then DIETERICI'S and RACIBORSKI'S figures for the osmotic pressure of saturated NaCl solutions, which are based on vapor pressure determinations, are much nearer the truth than BROWN'S figures for the same solution. Although the

difference between the two figures is very large, the former being to the latter as 3:1, inspection of the curves in PHILIP'S little book shows that at a concentration of 700 grams of sucrose per liter of solution, the observed pressure would be to the theoretical pressure calculated on the basis of solution volumes as 3:1. Moreover, the figures of RACIBORSKI for saturated LiCl solutions (80.7 per cent at 20° C.) were obtained by the same methods which DIETERICI used for NaCl; his results give to this solution an osmotic pressure of 965.3 atmospheres. This does not seem too high if NaCl has a value of 375 atmospheres; for NaCl at saturation at 18° C. is a 5.42 M solution, and LiCl a 13.3 M solution (SMITH'S *General chemistry*).

These pressures are of interest on account of their magnitude. If this measure is correct for the osmotic pressure of saturated lithium chloride, then the internal forces causing entrance of water into air-dry *Xanthium* seeds must be at the initial moment in the neighborhood of 965 atmospheres. It is as difficult as it is interesting to think of a saturated aqueous salt solution being as "dry" as an air-dry seed.

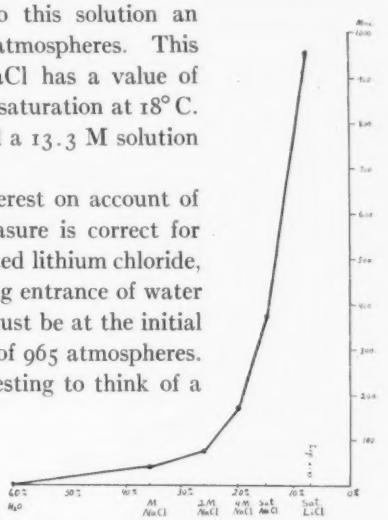

FIG. 8.—Curve of imbibition in M–NaOH, showing the development of osmotically active substances within the semipermeable coat during the second day; the pressure thus developed bursts the outer layers, but leaves the inner one intact.

From saturated solutions of NaCl these air-dry seeds will imbibe 7 per cent of their air-dry weight. It is readily seen, then, if the pressures given for the two solutions are correct, that the imbibition force in a seed which contains 8–9 per cent of hygroscopic moisture (air-dry) is 965 atmospheres, and that the addition of 7 per cent more water reduces the capillarity and imbibition force from 965 atmospheres to 375 atmospheres, a loss of 590 atmospheres.

The rapid increase of the internal forces with the decreasing water content of the seeds is shown graphically in fig. 8, which is

based upon the figures presented in this discussion. What the initial capillary or surface force would be if all hygroscopic water could be eliminated without changing the fundamental nature of the embryo must be left purely to conjecture; but the figures would probably exceed those which RODEWALD found for starch. He states that dry starch on swelling develops a pressure of 2523 atmospheres (**32,** p. 227). At present we have no adequate means of measuring such large forces.

III. Discussion

The results here recorded have a bearing upon many problems in widely separated fields of research. It will be impossible to consider every phase of the subject, and only the more important matters will be considered here.

The general occurrence of non-living semipermeable membranes in plant structures, especially as seed and fruit coats, is of the greatest interest. A large amount of work has been done upon seeds with coats intact, especially upon seeds showing delayed germination, in attempts to stimulate protoplasm to activity. FISCHER (**16**) used many different kinds of acids, alkalies, and salts on seeds. LEHMANN (**26**), BECKER (**6**), and many others have used Knop's solution, etc., as stimulants for resting seeds. In BECKER'S paper especially the seeds used by him, mostly Compositae, show such peculiar irregularities of behavior in germination that one cannot escape the conviction that the coats are responsible for much of it. If semipermeability of protective structures is as common as now appears to be the case, much of the work done with salts and other substances acting through the coats will lose considerably in its significance. The very salts which are assumed to enter the seed may be excluded by the testa of the seed. No safe conclusions can be drawn from studies in which the physical characters of the testa are not definitely known. This discovery only emphasizes what the writer has said elsewhere (**34**) regarding the necessity of removing seed coats when the properties of embryos are being investigated. The testa has physical and chemical characters which may enable it to modify greatly any factor entering into germination behavior, and the effects of these characters must be known before

any sound conclusions can be drawn. Semipermeability is now shown to be common enough that its existence should be proved or disproved before proceeding to use stimuli acting through membranes such as those referred to here.

With a number of the solutions used in these experiments a very high percentage of increase in weight was noticed, running much higher than the imbibition in pure water. This was noticed with $HgCl_2$, $AgNO_3$, several alcohols, acetic acid, and especially the alkalies. The intake in these cases cannot be considered as due entirely to imbibition and capillarity. Several things may be responsible for the large increase in weight. In the case of the solutions of the heavy metals their specific gravity has something to do with the great increase; but there is convincing evidence that this is not the only cause.

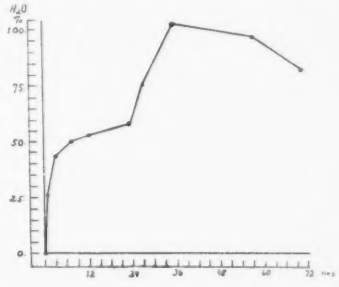

FIG. 9.—Curve showing increase of imbibition force in seeds with decrease of water content; percentage of H_2O is based on absolute dry weight; air-dry seeds are considered to have 8 per cent of hygroscopic moisture in this diagram; the force as here plotted is based on figures discussed in the text.

The seeds become greatly swollen, until in many instances they are perfectly cylindrical and stretched to inordinate size. In these cases a fluid is found between the coat and the rest of the seed. Evidently there is a dissolution of various constituents of the embryo whose decomposition products are osmotically active, which exert great pressure upon the coat from within, and which thus cause a very large intake of water. This is true especially when alkalies are used. Fig. 9 shows the curve of increase in weight of seeds kept in molecular NaOH. The earlier part of the curve, up to 24 hours, resembles the imbibition curve for water, only the entrance is a little more rapid. Then suddenly the weight increases under the pressure of dissolved organic substances from within, and by the end of three days the outer layers of the coat have been bursted, leaving the inner layer intact. Through its translucent cells one can see the

embryo, not much above normal in size, surrounded on all sides by a liquid which is held in by the semipermeable membrane.

This curve is of great interest in connection with FISCHER's (**16**) work on the seeds of water plants. When the seeds have a brittle instead of an elastic coat, the pressure from within may be great enough to rupture the coat, and allow oxygen or some other requisite for germination to enter. There is no doubt that this actually happens with acids and alkalies in the case of *Alisma Plantago-aquatica*, one of the seeds which FISCHER stimulated with hydrogen and hydroxyl ions, and which CROCKER (**15**) showed at the same time needed only to have the coat broken to bring about germination. In view of the effect of certain acids and alkalies on seeds with semipermeable coats, the reason for germination on treatment with these substances is obvious.

In this connection sight must not be lost of the possible effect of acids, alkalies, etc., on the colloids of the seed. FISCHER (**17, 18**) has shown the remarkable effect of acids and alkalies on the water-holding power of fibrin and frog mnscle, and has pointed out the relation of acids to edema and glaucoma (**19**). A part of the increased weight may be due to the colloids in the embryo holding more water in the different states of ionization induced in them by the presence of acids and alkalies; but most of it is certainly due to the osmotic pressure from within, which adds a large quantity beyond what capillarity and imbibition would take up.

The behavior of the coat in retaining dissolved substances adds further evidence to that already brought forward to show that even after prolonged treatment in tannin solvents the coats are still semipermeable.

The high internal forces in dry seeds is interesting in connection with recent work on the osmotic pressures of the cells of desert plants. FITTING (**20**) shows that desert vegetation has in many instances cell sap with more than 100 atmospheres of pressure, an adaptation which is of great importance to the plant whose "saturation deficit" must withdraw the water from an arid soil. The value of FITTING's work would have been enhanced if he had used boiling or freezing-point methods as a check to the plasmolytic methods of determining the osmotic pressure. The possibility suggests itself

that the forces which extract water from the soil particles and move it through the plant may not be entirely osmotic whenever the force needed runs high, but may involve capillarity and imbibition as well. A study of surface tension forces in soils should throw some light on this subject.

Many theories have been advanced to explain the cause of semipermeability. Chief among these are (1) the filter or sieve theory, (2) the solubility theory, (3) the "Haftdruck" theory of TRAUBE, and (4) the hydrone theory of ARMSTRONG. Each theory has a certain amount of supporting evidence, and any of them could be applied to the problems of physical or physiological semipermeability.

Since ARMSTRONG (**3**) used the hydrone theory to explain the semipermeability of the coat of *Hordeum vulgare* as described by BROWN, I have given it more attention than the others, not that it seemed to offer the best explanation, but because of its weakness.

ARMSTRONG'S conception of water as a mixture of hydrone, hydrol, and hydronol was developed several years ago (**1, 2, 4**). The hydrone compounds are composed of $=OH_2$, which is related to the hydrones much as $=CH_2$ is to the carbon compounds. The more complex molecules, as penthydrone, are believed to be inert, resembling the polymethylenes in this respect; whereas the simple hydrones are much more active.

The size of the hydrone molecule is believed to be influenced by temperature. As the temperature of water rises, the complex hydrones are supposed to split up into simpler molecules, which renders the water much more active in all its relations. This conception has received some support recently from the work of BROWN and WORLEY (**12**), who studied the relation of temperature to the rate of water imbibition by seeds of *Hordeum vulgare*.

Their results indicate that the velocity of water intake may be a logarithmic function of the temperature. The temperature coefficient for the imbibition rate follows approximately the VAN'T HOFF law, indicating that chemical processes are involved in water intake. They suggest that the chemical change thus indicated is the simplification of the complex hydrone compounds into smaller, more active molecules.

Certain solutes are believed to have the same, or similar, effects upon the hydrones as increase in temperature. For instance, acetic acid, and other substances mentioned in this paper as leading to supernormal imbibition, may increase the rate of entrance by breaking down the inert complex molecules of water. If the interpretation of the results of BROWN and WORLEY is correct, the measure of the rate of water intake may be looked upon as a measure of the activity of water when in a certain state which depends on temperature, or on the presence of certain solutes. While this work is of the greatest interest, it is too early to make any general applications of their results.

If the writer understands ARMSTRONG'S application of his hydrone theory (**3**) to semipermeable membranes, the fine particles which make up the membrane are assumed to be chemically united to hydrone or hydrol, perhaps under the influence of surface force. All the intramolecular passageways through the membrane are therefore guarded by hydrone elements. When a salt goes into solution the solute molecules also are believed to be hydrolated in case of non-penetrating solutes. Now if a hydrolated salt presents itself for passage through a hydrolated or hydronated membrane, the salt is seized and held back by the mutual attraction of the hydrolated surfaces of the salt and membrane. If on the other hand an unhydrolated salt presents itself to the same membrane the hydrolated passageways are indifferent to the salt, and it passes through the membrane without the "chemical seizure" retarding its entrance. Selective action would depend upon the salt rather than upon the membrane, for an unhydrolated membrane should allow all salts to pass providing other physical and chemical conditions necessary to passage were met.

The penetrating salts, whether electrolytes or non-electrolytes, are conceived to be those which can exist in water solution in unhydrated condition, or which attract water only to a slight extent; whereas those which are excluded are those which form hydration compounds of considerable stability; and the semipermeability depends on the mutual attraction of hydrated salt and hydrated membrane.

It is the writer's opinion that ARMSTRONG'S rather fanciful

theory of the structure of water has not been well received by American chemists; perhaps it has not been given the attention it deserves. As regards its application to the problems of semipermeability there seems to be some difficulty. The data obtained at one point with *Xanthium* seeds argue against the hydrone theory as a universal explanation.

For instance, BROWN found that both NaCl and $AgNO_3$ failed to penetrate the coat of *Hordeum*. ARMSTRONG says in effect that the membrane is hydronated, both salts are hydronated, therefore they do not enter, although the water in which they are dissolved may enter freely. In my experiments NaCl does not pass through the *Xanthium* coats, therefore both salt and membrane must be hydronated. Now if ARMSTRONG'S theory is correct, a solution of $AgNO_3$ should also be excluded, for BROWN'S experiments have shown that $AgNO_3$ is a hydronated salt when in solution. But this does not happen, for silver nitrate is retarded but slightly, whereas according to ARMSTRONG'S hypothesis the mutually hydronated surfaces should attract each other so strongly as to prevent the passage of the salt. Trichloracetic acid was exceptional in its behavior in BROWN'S experiments, so that the hydrone theory cannot as yet be accepted as a general explanation of selective semipermeability. More facts are needed before the physiological semipermeability of living or the physical semipermeability of non-living membranes can be adequately explained.

Further studies on the seed coats of *Xanthium* and other seeds are being continued. The microchemistry of the coat, its relation to oxygen diffusion in respiration, its relation to the impermeability of "dry" solvents, and its use as a measure of surface tension forces in soils are now under investigation. The earlier results of these studies cannot of course be presented at this time.

IV. Summary

1. The dry seed coats of *Xanthium* are impermeable to dry alcohol, ether, chloroform, and acetone. BECQUEREL'S results with the coats of other seeds are confirmed.

2. Evidence of the diffusion of oxygen through absolutely dry seed coats was not obtained.

3. Selective semipermeability like that found in *Hordeum* has been demonstrated for the seed coat of *Xanthium*.

4. The following substances seem to be excluded: NaCl, $CuSO_4$, K_2CrO_4, $Na_2S_2O_3$, glycerol, sugars, HCl, tartaric acid.

5. The following enter, either slowly or rapidly: NH_4NO_3, $AgNO_3$, $NaNO_3$, KNO_3, KCl, $HgCl_2$, $FeSO_4$, alcohols, ether, iodine, KOH, NaOH, H_2SO_4, HNO_3, acetic acid, lactic acid, citric acid.

6. The selective activity is independent of any living substance in the seed coat.

7. The coat of *Xanthium* can be removed and used as an osmotic membrane, possessing a great advantage over the coats investigated by BROWN and SCHRÖDER.

8. The testa is composed of three layers, the outer of which cannot function as a semipermeable membrane. The middle layer is several cells thick, the inner layer one cell thick except in the chalazal region. This last layer is probably the nucellar epidermis.

9. By use of strong alkalies the inner membrane can be split loose from the middle layer. Both layers possess osmotic properties, the inner layer in a higher degree than the middle one.

10. Neither layer is as efficient alone as when both are left together. The impairing of the membranes may be due to stretching, or to the effects of the macerating agent.

11. The inner layer is nearly pure cellulose, unsuberized, but perhaps containing some tannin. The middle coat contains more tannin than the inner coat.

12. The tannin does not form a continuous layer in the seed coat. Moreover, treatment with tannin solvents does not destroy semipermeability. The evidence is adverse to REICHARD'S view that semipermeability is due to tannin compounds.

13. Semipermeability has been demonstrated for the seed coats of a number of plants in six widely separated families. Many dead plant membranes may possess this property.

14. The capillary and imbibition force of the embryo of *Xanthium* as measured by the osmotic pressure of concentrated salt solutions is about 965 atmospheres when the seed is air-dry.

15. An increase in the moisture of the embryo equal to 7 per cent of its air-dry weight reduces the internal forces by 590 atmospheres.

16. The unusual intake of water noticed with certain substances, especially with certain acids and alkalies, is due largely to the development of osmotically active substances inside the semipermeable membrane.

17. There is some evidence unfavorable to ARMSTRONG'S hydrone theory of selective semipermeability.

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THE ORIGIN AND DEVELOPMENT OF THE EMBRYO SAC AND EMBRYO OF DENDROPHTHORA OPUNTIOIDES AND D. GRACILE. II

HARLAN HARVEY YORK

(WITH PLATE VII)

Embryo-formation in D. opuntioides

Following the arrangement of the nuclei in the micropylar end of the sac, as described above, they enlarge, the one toward the so-called polar nuclei becoming the largest. This nucleus becomes the one functional cell from which the endosperm and embryo are derived. Later the "synergid" and the polar nuclei begin to degenerate and in a short time disappear. Preparatory to division, the functional nucleus becomes very much enlarged and divides by ordinary mitosis, in a plane transverse to the longitudinal axis of the sac (fig. 46). This mitotic division was the first observed following that of the megaspore mother cell. It was not possible to count the chromosomes, but the number was apparently equal to that seen at the division of the megaspore mother cell. The two cells resulting from the division of the functional nucleus divide transversely to the plane of the first division. After a series of divisions a large oval mass of tissue is formed which occupies the upper end of the sac. The cells of this body are large and appear to be exactly alike (fig. 47). After this endosperm-like body has reached about one-twentieth of its size in the mature seed, the cytoplasm of one of the central cells becomes more densely granular than in the others. This cell constitutes the one-celled stage of the embryo (fig. 48). It divides by a wall almost transverse to the longitudinal axis of the flower, forming a 2-celled embryo (fig. 49). A division of these two cells in a plane transverse to the wall which separates them results in the formation of the 4-celled embryo (fig. 50).

The embryo thus arises indirectly from a single cell without fertilization having occurred. The author examined carefully more than 500 plants of *D. opuntioides,* occurring within a radius

of 5 or 6 miles around Cinchona, Jamaica, at altitudes varying from 2000 to 5500 ft. above sea-level, and could find no staminate flowers. Microtome sections of more than 200 ovules mature enough to show whether pollination had occurred gave no traces of pollen adhering to the stigmas or pollen tubes within the tissue of the style. Examination of 150–200 ovules of *D. gracile* likewise failed to show pollen tubes. If pollination does occur in these plants, the mode of origin of the embryo and endosperm is strikingly different from anything elsewhere known. In recent studies on *Phoradendron flavescens*, the author observed near Austin, Texas, a single plant more than a mile distant from others of its kind, fruiting abundantly. The berries of this plant contained normal seeds. Since *Phoradendron flavescens* is dioecious, the chances for wind pollination in this case were very slight. Hence it is apparent that this plant was producing fruit without having been pollinated. In the following year a number of branches bearing pistillate flowers were covered with cheese-cloth bags before the shedding of the pollen had begun, and allowed to remain until there was no longer any possibility of pollination. They produced fruit as abundantly as the uncovered branches. Thus it is evident that in *Phoradendron flavescens* pollination is not necessary for the production of seeds. In fact, pollination probably does not occur in plants under natural conditions, for in studying sections of a few hundred young fruits of this species, no pollen tubes or germinated pollen were seen. There are 18–22 chromosomes present in the cells of the embryo of *D. opuntioides*, which is the number found at the division of the megaspore mother cells. There is thus apparently a constant number of chromosomes throughout all the different stages of development of this plant, which seems clearly to preclude all possibility of fertilization. Since no nuclear fusions were seen within the sac, it seems evident that no reduction division has occurred previous to the formation of the gametophyte. Estimates of chromosomes made in the cells of the embryo of *D. gracile* show that there are 18–20. The cells of the endosperm in each species contain the same number of chromosomes as is found in the embryo.

It was impossible to obtain a complete series of good sections of the different stages in the development of the endosperm and

embryo, owing to shrinkage of the material from lack of penetration of the fixing fluids. The growth of the embryo proceeds very slowly as compared with the endosperm, and it is but a small spherical mass of cells when the latter has come to occupy the whole central region of the carpellary tissue (fig. 51). At first the embryo is intimately connected with the endosperm, from which it gradually separates as development continues. The micropylar end or the radicle is first freed, while the cotyledons are last to be separated. The embryo is strictly dicotyledonous, and when mature is almost entirely imbedded within the endosperm (fig. 52). The endosperm enlarges at the expense of the adjacent tissues. Soon after the origin of the embryo, it begins to elongate in the direction of the longitudinal axis of the ovary and at the same time becomes flattened in the plane of flattening of the spike. Growth at first is almost entirely upward and the apex of the endosperm reaches to the base of the style. Later, by gradually digesting the remains of the placenta, it advances downward into the plate of tracheids at the base of the ovary. The endosperm thus replaces the entire axial region of the flower and lies in direct contact with the vascular traces of the carpels (figs. 20, 21, *tc*) which later form a part of the covering of the seed (figs. 22, 66).

The endosperm is composed of large parenchyma cells, the walls of which are relatively thin and of a most uniform thickness. The cells of the external layer are largest, attaining their greatest size at the base of the endosperm (figs. 53, 54). They are not specially modified for protection as in *Phoradendron flavescens*, and do not form a true epidermis. They fit loosely together and seem to have an absorptive function. This is especially true in the basal portion of the endosperm, in which the outermost layer of cells contains large nuclei and very fine granular cytoplasm which stains brownish yellow in iodine (fig. 53). Since these cells are in direct contact with tracheid cells, the greater bulk of food passing into the endosperm must enter through them. Hence they are considered as being primarily absorptive in function. The fact that the cells of the outer layer fit loosely together also indicates that they are capable of readily absorbing water when germination begins. Sections of seeds which had just begun to germinate showed that

these cells had become turgid and crowded closely together into a very compact layer (fig. 55).

In the ripe seed the outer layer of cells contains large quantities of a fatty substance which stains black in osmic acid and dark blue in cyanin (figs. 53, 54). When germination begins, the fat, as indicated in figs. 53 and 54, almost entirely disappears from them and they become practically colorless. Large brown cystoliths, the chemical nature of which was not determined, were found in some of the cells. The remaining cells contain starch and other food substances which are more or less limited to certain portions. Fatty substances as seen in the outer cells also occur in the inner region of the endosperm and are in greatest abundance in the chalazal part (fig. 53). There is a single layer of cells, densely filled with chloroplasts and starch, subjacent to the outer cells (fig. 55, *cl*). The cells adjacent to the cotyledons (fig. 55, *i*) contain practically no chlorophyll and very little starch, but substances which stain like proteins. Immediately above this layer the cells are abundantly filled with starch (fig. 55, *z*). The remaining portion contains less starch and chlorophyll, but in addition has large cystoliths of calcium oxalate (fig. 55, *cs*). The cells of the embryo are also well supplied with chlorophyll. When germination begins, the chlorophyll present in the seed becomes greatly intensified. It is important to note in this connection that chlorophyll is most abundant in the cells just below the outer layer of cells which become almost colorless when germination occurs. The chlorophyll in the endosperm is manifestly for photosynthetic work, as a result of which food is furnished to the young plant until it becomes fixed on its host. It becomes more evident that the chlorophyll performs the work of photosynthesis from the fact that the seeds will not germinate in the dark nor will germinating seeds continue to develop if kept from the light. A number of attempts to germinate seeds of *D. opuntioides* in a dark chamber in the laboratory were made, but all failed. Also small dark chambers were constructed out of black paraffined paper over seeds that had been dropped on the branches by birds. At the end of four weeks these seeds had not germinated, while uncovered seeds near those which were covered had germinated. Seeds of *Den-*

dropemon parvifolius were placed in the dark chamber with those of *Dendrophthora opuntioides* and germinated as readily as those under normal conditions. Chlorophyll is not present in the endosperm of *Dendropemon parvifolius*, but is abundant in the embryo. From this fact it is seen that the endosperm in *Dendrophthora* and also in *Phoradendron*, which is quite similar, is also a carbon dioxide assimilating organ. It will be of interest to mention here that the radicles of the seedlings of *Dendropemon parvifolius* which germinated in darkness did not turn down toward the substratum, but grew out at various angles. Some, for example, extended upward at an angle of 90°, while others were parallel to the substratum. Thus the bending of the radicles toward the substratum is not due to gravity or to attraction by the substratum, but their behavior seems to be a negative response to the stimulus of light.

DUTROCHET (**6**) found that the "hypocotyls" of *Viscum album* are negatively heliotropic. His observations were later confirmed by WIESNER (**39**). The endosperm in its development, as seen in this study, may thus be regarded as being parasitic, drawing its nourishment through its outer layer of haustorial cells from the adjacent tissues. It also becomes a storage tissue, containing an abundance of food material for the embryo and a region in which photosynthesis occurs.

The viscin

Owing to the lack of fresh material at Baltimore, a detailed study of the viscin has not been possible. Its structure is essentially the same as described and figured by JOHNSON in *Arceuthobium Oxycedri*, and by PEIRCE in *A. occidentale*. Parallel with the maturation of the embryo sac, the cells in the outer half of the carpels become colorless and begin to enlarge (figs. 20, 56). Later they elongate obliquely toward the base of the style when the formation of the embryo begins. In the ripe berry they are greatly elongated and their walls consist of two layers. The outer layer is composed of gelatinous material, while the inner is usually made up chiefly of spiral cellulose thickenings (fig. 57). As a rule the cell cavity is very narrow, and in some cases has been almost obliterated, the cell being chiefly composed of gelatinous material (figs. 58, 59). In some cells the outer wall is spirally thickened.

The inner ends of the cells are attached to a sort of membrane which is two or three cell layers in thickness. PEIRCE has described a similar structure, a "sclerotic membrane," in *Arceuthobium occidentale* as being composed of three or four layers of tannin cells. Apparently there was no tannin in the cells of this body in *Dendrophthora opuntioides*. The viscin in *Phoradendron flavescens* presents essentially the same structure as that here just described, though this was not recognized by the author in his earlier studies on this plant. The layer of viscin surrounding the seed in *Dendrophthora* is much less in proportion to the size of the berry than in *Phoradendron flavescens*. In the latter the viscin evidently prevents extreme drying out and is useful in absorbing water before germination begins. It is not necessary for the germination of the seeds, but if it is stripped from them they will dry up in a few weeks' time when exposed to a dry atmosphere. Seeds from which the pulp has not been removed retain their vitality for an indefinite period of time. In January 1909, the author collected in Austin, Texas, berries of *Phoradendron flavescens* which were dried at room temperature. During June 1910, 12 of the dried berries were soaked in water for 24 hours, after which a part of the pulp was removed and the seeds were placed in a moist chamber. At the end of two weeks 4 of the seeds had begun to germinate. Owing to the warm moist environment of *Dendrophthora opuntioides*, the pulp is probably not an important factor as a means of protection and aid in germination of its seeds, since they germinate almost immediately after being deposited.

The mature berries are bright red in color, oval in shape, 7–9 mm. long, and 6–8 mm. in diameter. Owing to the peculiar order of origin of the flowers, various gradations in the development of fruits, from flowers to ripe berries, often occur in the same spike. The seed is enveloped by a thin layer of viscin, which is surrounded by a thick fleshly pericarp. The latter is covered by a heavily cutinized single-layered epidermis (figs. 22, 66).

Embryo-formation in D. gracile

The micropylar nuclei of the sac enlarge as much as they do in *D. opuntioides*. Meanwhile the two polar nuclei, which are

located some distance below them, are fusing and migrating upward in the sac (fig. 60). The nucleus resulting from their union lies just below the egg and becomes the nucleus of the primordial cell of the proembryo (figs. 61, 62). Before division it enlarges greatly and is apparently cut off from the sac below by a transverse wall. At the same time two of the micropylar nuclei, the synergids, begin to degenerate. The other nucleus, which is homologous with the one in the sac of *D. opuntioides* from which the proembryo originates, continues to enlarge for a short time as if preparing to divide, and then also begins to degenerate before division of the fusion nucleus occurs. The primordial cell of the proembryo divides by ordinary mitosis (figs. 62, 63). The two resulting cells (fig. 64) by further divisions form a proembryo, which is similar to that of *D. opuntioides* (fig. 65). The development which now follows is also the same as has been given above, hence further descriptions are unnecessary. The origin of the embryo as seen in *D. gracile* suggests the example described by FARMER and DIGBY (**8**) in *Lastrea pseudo-mas* var. *polydactyla* Wills, in which the embryo arises from a nucleus resulting from the union of two body cells of the prothallus which has come from a normally developed spore and has the haploid number of chromosomes. The difference in the mode of origin of the embryos of *D. gracile* and *D. opuntioides* also recalls that shown by the above mentioned authors between *Lastrea pseudo-mas* var. *polydactyla* Wills and the closely related *Lastrea pseudo-mas* var. *polydactyla* Dadds. In the latter the embryo arises from a projection or budding out of the prothallus which has arisen aposporously. In this same variety these authors also found nuclear fusions occurring in the prothalli which had come from normal spores, quite similar to those in the variety first mentioned. It is probable that a phenomenon similar to that just stated for Dadds' fern occurs in *D. opuntioides*, though no evidence for such a supposition was seen. Owing to the lack of material, it was not possible to make a detailed study of the division of the megaspore mother cell. At the division of the microspore mother cell in *D. gracile* 9 chromosomes were found passing to each pole of the spindle; 18–20 chromosomes were estimated in the cells of the developing embryo. Apparently the same number is also present

in the nuclei of the endosperm. From these facts it is clearly evi dent that fusion of the polar nuclei in *D. gracile* indicates that the nuclear behavior in the origin of the female gametophyte is the same as in an ordinary sexual plant.

Discussion

The embryos of *D. opuntioides* and *D. gracile* arise asexually. In the former, reduction division does not occur preceding the formation of the gametophyte, the nuclei of which thus contain the diploid number of chromosomes. The gametophyte is aposporous in origin. Examples of apospory in seed plants have been described by JUEL (**16**) in *Antennaria alpina*, by MURBECK (**22**) and STRASBURGER (**32**) in certain species of *Alchemilla*, by TREUB (**34**) in *Balanophora elongata*, by OVERTON (**27**) in *Thalictrum purpurascens*, by LOTSY (**19**) in *Balanophora globosa*, by OSTENFELD (**25, 26**), ROSENBERG (**29, 30**), and MURBECK in species of *Hieracium*, by WINKLER (**40, 41**) in *Wikstroemia indica*, and by RAUNKIAER (**28**), MURBECK (**23**), and JUEL (**17**) in a number of forms of *Taraxacum*. Apogamy is associated with the aposporous origin of the gametophyte in these forms. In each of these plants, with the exception of *Balanophora*, the embryo arises from what is to external appearances an egg. From a cytological point of view the studies of STRASBURGER and WINKLER are the most important. The former regarded the cell giving rise to the embryo as a sporophytic cell, since it possesses the diploid number of chromosomes. According to this view the embryo may be said to develop by vegetative budding. STRASBURGER regards the process as a case of apogamy and not parthenogenesis as some authors claim. He defines an egg as a cell having the haploid number of chromosomes and capable of being fertilized.

WINKLER disagrees with STRASBURGER in regard to the use of the terms apogamy and parthenogenesis and the theoretical significance of the chromosomes. According to him, apogamy is the origin of a sporophyte from some cell or group of cells of the gametophyte other than the egg. He uses the term parthenogenesis to indicate the formation of an embryo, developed without fertilization, from a cell having the position of an egg, whether the nucleus

of this cell is haploid or diploid. WINKLER also maintains that other factors besides the number of chromosomes determine the character of the gametophyte as well as the sporophyte. YAMANOUCHI'S (**42**) studies on apogamy in *Nephrodium* support this hypothesis. If we accept STRASBURGER'S narrow definition of the egg, it seems logical to restrict the term gametophyte to structures having the haploid number of chromosomes, that is, to bodies developed after a normal sporogenesis. Regarding *Dendrophthora opuntioides* from this point of view, a true megaspore formation does not occur, the gametophytic generation itself is omitted, and the nuclei of the embryo sac are thus really vegetative or sporophytic in character. If the egglike cell in *Dendrophthora*, as well as in other similar cases, is a vegetative or sporophytic cell, it is strikingly different in behavior from all other sporophytic cells which we know.

I find no record among seed plants of the origin of an embryo from a cell of an endosperm-like mass, which like that of *Dendrophthora* has been derived from a single egglike cell. If this cell is really a vegetative cell of the sporophyte, we should expect it to develop directly into a sporophyte, as nucellar cells do in *Coelebogyne* and *Funkia*. In *Antennaria alpina* (JUEL **16**) or *Thalictrum Fendleri* (DAY **4**), for example, the egg cell which develops without fertilization should, if it is really a sporophytic cell, give rise to a sporophyte having the same sex as its parent. Furthermore, the derivation of the endosperm from a single polar nucleus is a peculiar phenomenon if this nucleus be regarded as sporophytic in character.

In the ferns, aposporous prothalli whose nuclei contain the sporophytic number of chromosomes are distinctly different in structure and in possessing sex organs from the parent sporophytes. Hence chromosome number cannot be the sole morphogenic factor in the nucleus. If the nucleus in any way determines structure, it is evident that the nuclei of an aposporous embryo sac must differ in some manner from those of the parent sporophyte, in spite of the identity in chromosome number. Morphologically the nuclei of such an embryo sac may be said to be equivalent in the same sense that the nuclei of a typical embryo sac are. The nucleus from which the embryo sac of *Dendrophthora opuntioides* develops,

however, is not a mere vegetative one, but is potentially distinct from all other nuclei in the sac. The functional nucleus in the sac of *D. opuntioides* occupies the position of an egg in the usual type of embryo sac. It is different from a diploid egg in that it does not give rise directly to an embryo. It may thus be regarded as a pseudo-egg and the tissue developing from it may be considered a proembryo or pseudo-endosperm. Owing to the very wide range in meaning of the term endosperm, it is perhaps allowable to designate the proembryo as endosperm, although it is not endosperm in the strict sense. TREUB in his studies on *Balanophora elongata* found that one of the polar nuclei gives rise to a mass of tissue which is quite similar to the proembryo described above. He called this body endosperm. The embryo arises from one of the central cells of this body as in *Dendrophthora*. He asserts that the embryo is apogamous in origin. LOTSY arrived at the same conclusions from his studies on *Balanophora globosa*. WINKLER, agreeing with TREUB, designates this mode of origin of the embryo as "somatic apogamy," since the nuclei of the endosperm possess the diploid number of chromosomes. WINKLER applies this same term to the mode of origin of the embryo, for example in that of the *Lastrea pseudo-mas* var. *polydactyla* Dadds, in which the embryo arises by a budding out of the prothallus. Since in *D. opuntioides*, as well as in *Balanophora*, the embryo does not arise directly from the gametophyte, it may be spoken of as pseudo-apogamous in origin.

The fact that there is a fusion of two nuclei in the sac of *D. gracile* preceding the formation of the proembryo is presumptive evidence that warrants the assumption that reduction has taken place in the formation of the megaspores. The nuclei of the sac have thus the haploid number of chromosomes. The nucleus occupying the position of an egg may or may not be capable of being fertilized. The two nuclei which occupy the position of polar nuclei are, as in *D. opuntioides*, derived from the same nucleus. Such a fusion of sister nuclei is a phenomenon of unusual occurrence among plants. It is not unknown among animals. BRAUER (**3**) has shown that in the majority of parthenogenetic eggs of *Artemia salina* only one polar body is formed; a second one is occasionally

produced. This one, however, never passes out of the egg, but after migrating a short distance its nucleus returns to the egg nucleus and fuses with it, thus restoring the diploid number of chromosomes. BLACKMAN (**2**) believes that the nuclear fusion occurring in the aecidium of *Phragmidium* is a "reduced form of fertilization." The fusion of two gametophytic nuclei in *D. gracile* is indeed quite comparable to the union of the nucleus of one prothallial cell with that of another in *Lastrea pseudo-mas* var. *polydactyla* Wills, which FARMER and DIGBY designate as pseudo-apogamy. They state:

> We would suggest the term pseudo-apogamy to cover instances in which the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which morphologically are not sexually differentiated. This would include the process as it occurs in the Uredineae and probably the Ascomycetes, and also the growing number of instances of apogamy in prothallia induced by cultural conditions, as well as such cases of obligate apogamy as that of the *polydactyla* varieties of the male fern.

According to them, conjugation of adjoining cells of the same filament in *Spirogyra* might be looked upon as examples of an analogous phenomenon. Whether the nuclei which fuse in *D. gracile*, in *Lastrea pseudo-mas* var. *polydactyla* Wills, and in *Spirogyra* really are undifferentiated morphologically is not definitely known. The gametes of *Spirogyra* or *Mucor*, for example, are believed to be morphologically alike. Aside from the fact that they are sometimes produced on different individuals, does not their conjugation constitute a process essentially like the examples just mentioned? Physiologically the gametes of *Spirogyra* and *Mucor* are believed to differ distinctly from each other. So in *D. gracile*, the author regards the two gametophytic nuclei which conjugate as potentially different from each other and from all other nuclei in the gametophyte. Externally they are alike. Therefore, may not their fusion constitute a process essentially like that of the conjugation of the gametes in *Spirogyra?* The union of the gametophytic nuclei in *D. gracile* does not result in the mixing of parental characters from separate individuals and does not constitute a true sexual process in this respect. Their fusion results in doubling the number of chromosomes and furnishes a stimulus to further development.

In these respects this process may be said to be like the usual type of fertilization. This conjugation of gametophytic nuclei in *D. gracile* may also in a sense be regarded as a sexual process, in that there is a gametophytic generation followed by a sporophytic in which the sexes are in different individuals.

DEBARY (**5**) was the first to use the term apogamy, restricting it to cases in which fertilization had disappeared;[1] hence this interpretation does not apply in *D. gracile*. The union of the polar nuclei in this plant should be regarded as a substitution process or pseudo-fertilization, which in some respects is like a true fertilization. The body arising from the fusion nucleus represents a proembryo or pseudo-endosperm, and the embryo is regarded as pseudo-apogamous in origin, as in *D. opuntioides*. The term pseudo-apogamy is not used here in the sense in which it was used by FARMER and DIGBY. In *Lastrea pseudo-mas* var. *polydactyla* Wills, there is a fusion of nuclei which the author believes is a substituted form of fertilization quite like that in *D. gracile*.

The presence of such a difference in the mode of origin of the proembryo in two closely related species as shown here is indeed a very striking phenomenon; perhaps, however, no more so than the two different modes of origin of the sporophyte in the varieties of *Lastrea pseudo-mas* already mentioned. This phenomenon may be due to the difference in the number of chromosomes present in the gametophytic cells. The fact that in one example development occurs without any sort of fertilization, while in the other it does not take place until the number of chromosomes is doubled, supports the theory that a nucleus must have the sporophytic number of chromosomes for starting the sporophytic generation. YAMANOUCHI's account of the origin of a sporophyte whose cells contain the haploid number of chromosomes from a haploid gametophyte in *Nephrodium* is evidence against this hypothesis. One of the most important problems for solution is the determination of the factors which have led to this difference. Since we know comparatively little of the physiological relation of the Loranthaceae to their hosts, it is almost useless to attempt an explanation of this phenome-

[1] "Dass einer Species (oder Varietät) die sexuelle Zeugung verloren geht und durch einen andern Reproduktionsprozess ersetzt wird."

non. *D. opuntioides* and *D. gracile* usually occur on different species of plants, hence the food substances which they absorb are probably different in some respects. This difference in the character of food material may have given rise to the difference in the development of these two parasites. In view of the facts just mentioned, such an interpretation seems quite allowable, since the type of reproductive organs formed in *Saprolegnia* has been shown to depend upon the character of the medium in which it is grown.

Summary and conclusions

The general plane of branching of *Dendrophthora opuntioides* and *D. gracile* is isolateral.

The flower originates a short distance above the axil of the subtending bract.

The floral axis elongates, filling the entire ovarian cavity, and does not become attached to the carpels.

The ovules are greatly reduced, being practically naked nucelli, which are borne on a central placenta. There are two nucelli oppositely placed in the plane of flattening of the "mamelon." In a number of examples vestiges of integuments were found.

A single archesporial cell, hypodermal in origin, arises in each nucellus. The archesporial cell becomes the megaspore mother cell, which gives rise to two megaspores. The one toward the apex of the "mamelon" gives rise to an embryo sac.

Following the formation of the 4-nucleate stage of the embryo sac, the micropylar end of the sac grows almost straight downward until it extends below the level of the insertion of the placenta. It then curves outward into the tissue of the carpel, bends, and grows upward beneath the lining layer of cells of the ovarian cavity until the end of the sac lies almost above the apex of the "mamelon." The embryo sac is hook-shaped; the short arm of which lies within the "mamelon," the long arm in the tissue of the carpel. The short arms of the embryo sac fuse, forming one continuous tube.

There are 7 or 8 nuclei in each sac, 2 antipodals in the chalazal end of the sac, 3 or 4 in the egg apparatus, and 2 polar nuclei.

The 2 antipodal nuclei are sister nuclei and were originally the

2 nuclei present in the chalazal end of the 4-nucleate sac. The 2 polar nuclei are sister nuclei and are derived from one of the 2 nuclei present in the micropylar end of the 4-nucleate sac. The nuclei of the egg apparatus arise from the sister nucleus of the one from which the polar nuclei are derived.

The nuclei of the 2, 4, 7, or 8-nucleate stages of the embryo sac are apparently formed amitotically.

Pollination does not occur.

An embryo is formed in only one of the two sacs in each flower. In *D. opuntioides* the egg nucleus gives rise to a mass of tissue, the proembryo. From a central cell of the proembryo the embryo develops. The endosperm is derived from the remaining cell of the proembryo.

The embryo is regarded as pseudo-apogamous in origin.

In *D. gracile* the "polar nuclei" fuse and give rise to a proembryo quite similar to that in *D. opuntioides*. The embryo and endosperm arise in the same manner as in the latter.

The embryo is strictly dicotyledonous and is almost entirely imbedded in the endosperm.

Apparently there is no chromosome reduction preceding the formation of the megaspores in *D. opuntioides*, while in *D. gracile* this process does seem to occur. In the latter, the diploid number of chromosomes is restored at the fusion of the "polar nuclei."

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EXPLANATION OF PLATE VII

FIG. 46.—Longitudinal section of two-celled proembryo; ×150.

FIG. 47.—Longitudinal section of proembryo; ×150.

FIG. 48.—Longitudinal section of proembryo of *D. gracile*, showing one-celled embryo; ×150.

FIG. 49.—Longitudinal section of proembryo with two-celled embryo; ×150.

FIG. 50.—Longitudinal section of proembryo with four-celled embryo; ×150.

FIG. 51.—Outline of longitudinal section of endosperm with inclosed embryo of a young seed, taken from young berry about the size of berry *A* in fig. 66; ×26.

Fig. 52.—Outline of longitudinal section of endosperm with embryo from mature seed, showing distribution of oil globules; ×17.5.

FIG. 53.—Portion of longitudinal section of endosperm from mature seed taken at *B* in fig. 52; ×150.

FIG. 54.—Portion of longitudinal section of endosperm from mature seed taken at *A* in fig. 52; ×150.

FIG. 55.—Portion of longitudinal section of germinating seed taken in region *C* in fig. 52; *cl*, cells abundantly supplied with chloroplasts; *cs*, cells containing cystoliths of calcium oxalate, starch, and small quantities of chloroplast; *z*, cells filled with starch; *e*, cells filled with protein-like substances; *ct*, cells of cotyledons of embryo; ×150.

FIG. 56.—Part of longitudinal section of carpel taken at *O* in fig. 20, showing cells (*vi*) which give rise to viscin; ×150.

FIGS. 57–59.—Portions of cells of viscin from ripe seed; ×150.

FIG. 60.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing fusion of polar nuclei; ×150.

FIG. 61.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing nucleus (*d*) formed by fusion of polar nuclei; disintegration of egg and synergid just beginning; ×150.

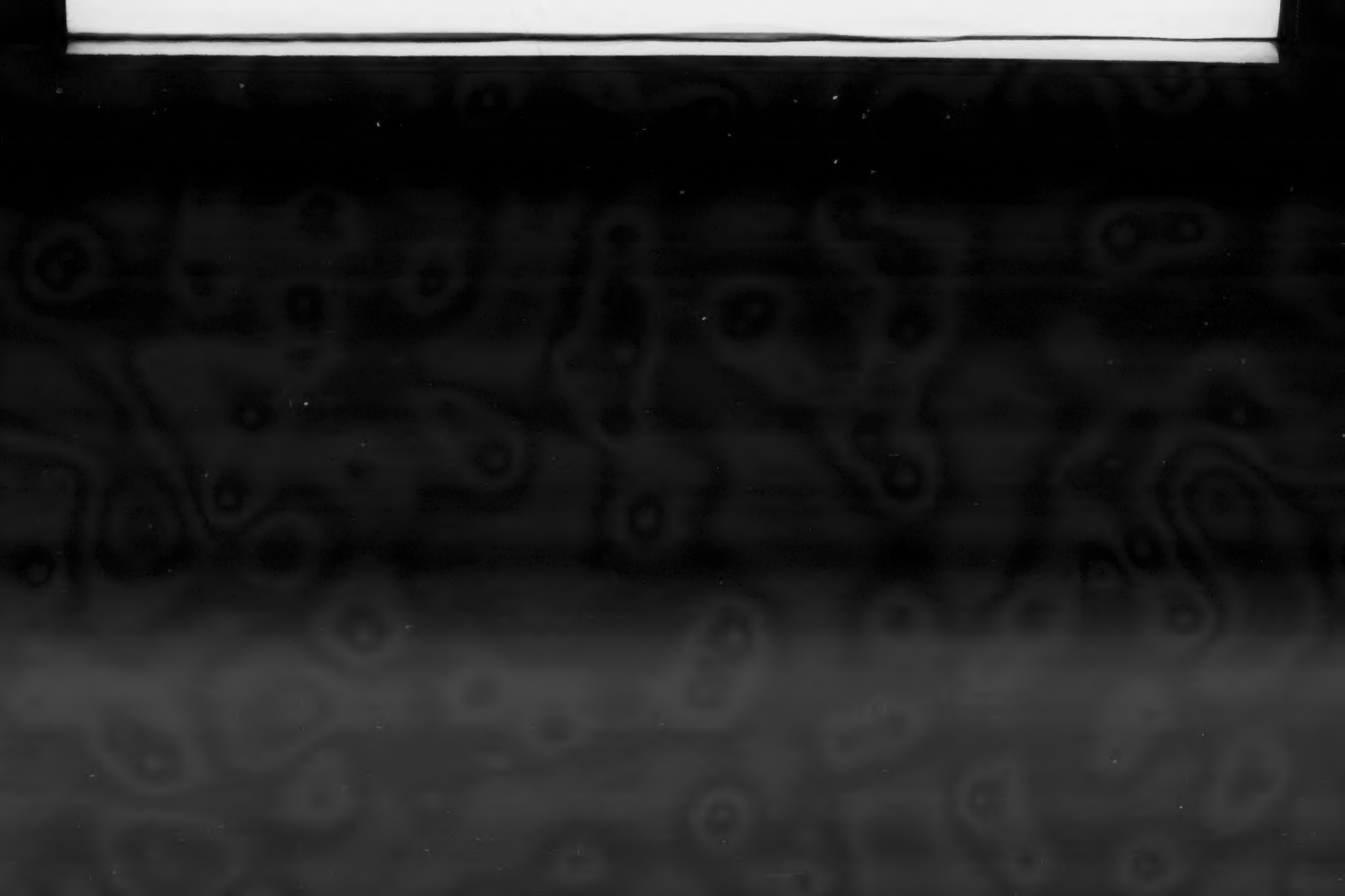
FIG. 62.—Same as fig. 61, with fusion nucleus (*d*) of *D. gracile* preparing to divide; synergids and egg disintegrating; ×150.

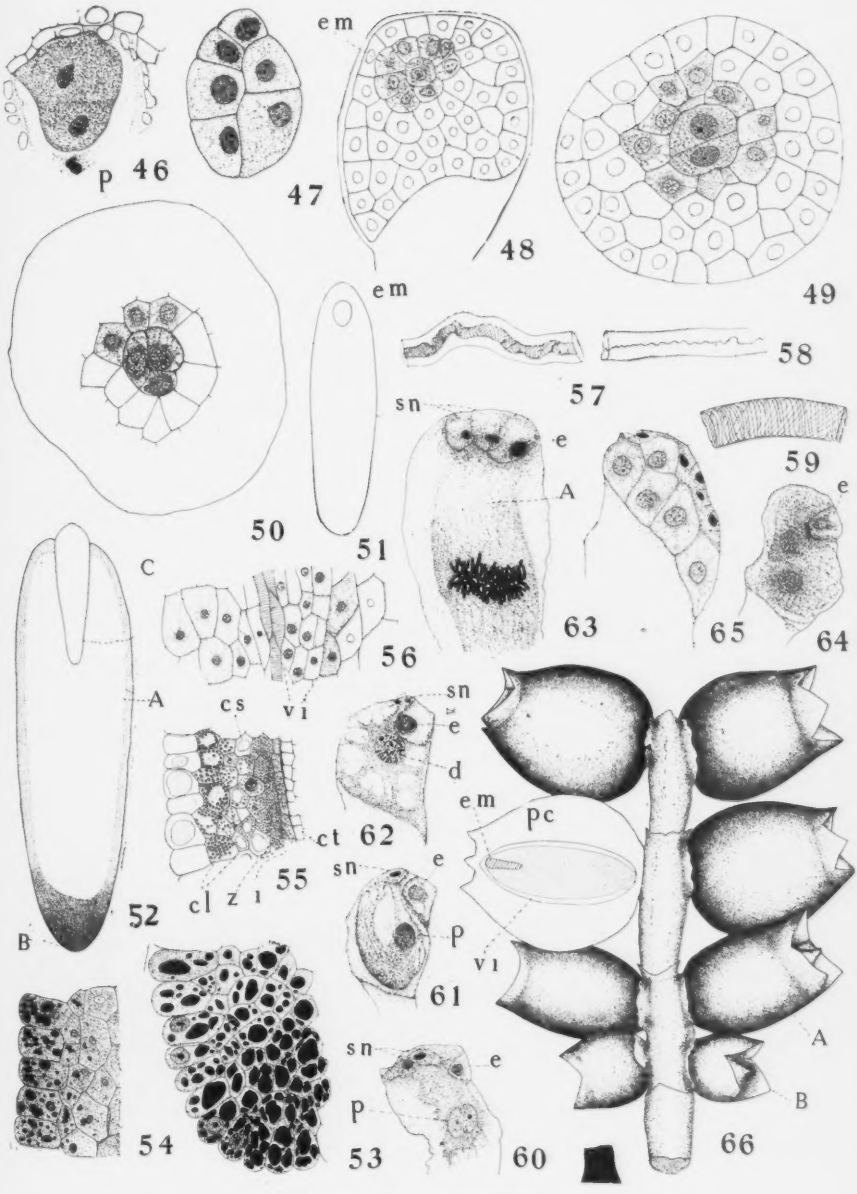
FIG. 63.—Division of fusion nucleus of *D. gracile:* probably displaced downward, from about level *A*, by fixation; the synergids and egg are disintegrating; ×250.

FIG. 64.—Two-celled embryo of *D. gracile:* synergids have disappeared; egg disintegrating; ×150.

FIG. 65.—Portion of longitudinal section of proembryo of *D. gracile*, which was composed of about 16 cells; ×150.

FIG. 66.—Lateral view of spike with berries in various stages of development; also showing position of endosperm and embryo in relation to the system of arrangement of different members of plant; *B*, flower in which formation of proembryo has just begun; ×2.8.





YORK on DENDROPHTHORA

XENIA AND THE ENDOSPERM OF ANGIOSPERMS

E. M. EAST

As is well known, the term "xenia" was proposed by FOCKE to describe any effect of pollen of another race upon the tissue of a seed plant apart from that initiating the formation of an embryo. As it has been exceedingly questionable whether any such effect beyond a chemical irritation ever occurs, the word has come to be applied to the appearance of the F_1 hybrid endosperm produced by the fusion of the second male nucleus with the so-called endosperm nucleus of the embryo sac, when its characters are different from those exhibited by the mother plant after self-fertilization.

Since the fact of this fusion was proved cytologically by GUIGNARD (10) and NAWASCHIN (11), data on this type of xenia have interested botanists because of the differences of opinion existing concerning the phylogenetic significance of the angiosperm endosperm.

The most detailed observations on xenia have been those on maize, since numerous maize varieties exist with differences in endosperm characters. The behavior of the following factors in heredity is known from the researches of DEVRIES (5), CORRENS (2), WEBBER (12), EAST and HAYES (7), and EMERSON (9). In addition, EAST (6) has found good indications of at least three additional factors that modify the expression of the red and the purple aleurone colors.

Factor	Action
S	Causing full development of starch grains
Y_1	Causing yellow color throughout endosperm
Y_2	Similar to Y_1 but not allelomorphic to it
C	Basic color factor necessary for color in aleurone cells
R	Present with *C* gives red color in aleurone cells
P	Present with *R* and *C* gives purple color in aleurone cells
I	Inhibits aleurone color when present with *RC* or *PRC*

Observations on crosses wherein these characters have been concerned have made it possible to formulate the following law regarding xenia:

When two races differ in a single visible endosperm character in which dominance is complete, xenia occurs only when the dominant parent is the male; when they differ in a single visible endosperm character in which dominance is incomplete or in two characters both of which are necessary for the development of the visible difference, xenia occurs when either is the male.

It is evident that such a statement can be true only if the two male nuclei always carry the same hereditary factors and if a male nucleus always enters into the formation of the endosperm. The first requirement has been satisfied in every experiment thus far recorded; the second requirement will now be considered.

In particular cases where xenia has followed the crossing of races differing in endosperm color, aleurone color, or ability to mature starch grains, the seeds are not uniform in appearance. One may be half starchy and half wrinkled; another may be half yellow and half colorless; still another may have half of the aleurone cells red or purple and the other half colorless. Examples of this kind are rarely found, although it is a common thing to find seeds with a mottled appearance affecting only the aleurone colors.

CORRENS and WEBBER suggested independently that in these cases the male nucleus may fail to unite with the fusion nucleus and each divide independently, forming either the half-and-half seeds or those which are mottled. WEBBER also suggested, as an alternate hypothesis, the fusion of the male nucleus with one of the polar nuclei, the other polar nucleus remaining independent and dividing.

EAST and HAYES have shown that CORRENS and WEBBER were dealing here with two phenomena. The seeds that are mottled become so only from the development or non-development of color in the aleurone cells. They merely exhibit irregularity of Mendelian dominance, since in some crosses practically all seeds heterozygous for one of the factors producing aleurone color are mottled, although homozygotes are fully colored. Furthermore, the mottling does not extend to the color or other character of the deeper endosperm tissue in case the parental varieties had such differences, which necessarily would be the condition if the endosperm had been formed according to either of WEBBER'S independent development hypotheses. This criticism has also been made independently by EMERSON (**8**).

The other cases, where the endosperm is divided more or less equally into two types, remain to be explained. The hypothesis of independent development of the male nucleus seems improbable if one may judge from relevant cytological data on both animals and plants. The second hypothesis is very plausible. As a third possibility, EAST and HAYES have suggested ordinary "endosperm fertilization" with subsequent vegetative segregation similar to that occurring in bud sports. This could be proved, according to them, if among the F_1 seeds of a cross between parents differing in two allelomorphic pairs, individuals should be found in which the parental characters were combined differently. No such cases have been recorded.

The difficulty of deciding between the first and the second hypothesis of WEBBER lies in the fact that individuals of this kind are very rare, and when they have been found the investigator has not been able to say which particular endosperm character was carried by the male cell and which by the female cell. This was because they have occurred in selfed hybrids where both pollen and egg cells were segregating various Mendelian factors. In the experiments now to be described, this difficulty has been overcome.

The red color in the aleurone cells of maize is due to the interaction of two factors that may be represented by the letters *C* and *R;* this color may be changed to purple by the presence of a third factor *P*. Red is *RC* and purple is *PRC*, therefore, although it must be understood both that other factors which have never been lost in any variety may enter into the combination, and that other factors which have been lost in certain varieties may affect the development of color.

Six homozygous white varieties may exist with the following zygotic formulae: *PPRRcc*, *PPrrcc*, *PPrrCC*, *ppRRcc*, *pprrCC*, and *pprrcc*. Any cross between these varieties of such a nature that *R* and *C* or *P*, *R*, and *C* are brought together results in the red or the purple color respectively.

Among the selfed maize ears that had been produced in the course of the writer's experiments were a number giving red wrinkled and white wrinkled seeds in the ratio of 3:1. These white seeds must have either the formula *ppRRcc* or *pprrCC*.

White seeds from three such ears were planted in isolated plots and used as male parents on the flowers of plants arising from *white* seeds found on selfed ears of 13 other families. A number of these families had the proper formulae to produce color, and about 60,000 red or purple seeds were produced. There were all-purple ears and all-red ears in several families. Other combinations gave purple and white seeds or red and white seeds in the ratio of 1:1. How this came about is clear if one assumes either of the formulae given above for the male parent. Suppose the male parent had the formula *ppRRcc:* a family with the formula *pprrCC* gives all-red ears, while one with the formula *pprrCc* gives ears with red and white seeds in the 1:1 ratio; a family with the formula *PPrrCC* gives all-purple ears, while one with the formula *PprrCC* or *PPrrCc* gives ears with purple and white seeds in the 1:1 ratio.

Considering first only the all-purple and the all-red ears, one must conclude that the fusion of the "endosperm nucleus" and the second male nucleus always occurs. If it did not occur, white seeds would result, because a factor from each parent is essential for the production of color.

Among these 60,000 seeds, 6 were found that showed the half-and-half condition; that is, color had developed on one side and not on the other. They were typical illustrations of the phenomenon which WEBBER'S two hypotheses were devised to explain. They occurred in only 0.01 per cent of the fertilizations, but in spite of their rarity they show that WEBBER'S first hypothesis, assuming independent development of the male nucleus, is untenable, since independent development of the paternal and the maternal nuclei could produce no color. No decision can be made between WEBBER'S second hypothesis—fusion of the male nucleus with one polar nucleus and independent development of the other—and the hypothesis of vegetative segregation after partial development. The bilateral symmetry of the halves of the seeds with and without color favors WEBBER'S idea; at the same time, it must be pointed out that the frequency of the occurrence is not too great to compare favorably with the frequency with which "bud sports" originate. Though it would afford some satisfaction, a precise explanation of these rare aberrations is not a necessary requisite

to several conclusions indicated by the experiments. It is evident that in the varieties of maize used, a paternal and a maternal nucleus carrying the same hereditary factors as are borne by the true gametes—in the case of the 7 factors investigated—always fuse in the formation of the endosperm. For this reason geneticists investigating maize have been correct in treating the endosperm as if it were an embryo. The endosperm characters have behaved exactly like plant characters. Two white varieties of sweet peas may carry factors both of which are necessary for the production of color. When they are crossed, color develops. Color develops in maize in a quite similar manner when the two complementary factors are carried by the "endosperm nucleus" and the second male nucleus. Nevertheless, one should keep in mind that the problem is complicated. COLLINS (1) found a white ear of maize in a yellow variety that behaved as if its seeds were crossed with the yellow. He interpreted the phenomenon as a mutation showing reversal of dominance, although the data on succeeding generations corroborated those obtained by previous investigators in which yellow was partially or completely dominant. It is not unlikely, however, that COLLINS merely happened upon a plant from white seed in which the male nucleus did not enter into the formation of the endosperm, although other interpretations are possible. This may seem like an odd statement after having shown that the two nuclei always fuse, but it is made advisedly. In most varieties of maize the two nuclei do appear always to fuse, but HAYES is now working out the details in a cross in which a Mexican starchy corn is one of the parents where the nuclei appear never to fuse. In other words, it seems that there may be varieties of maize in which endosperm formation is the opposite of that just described, and within each category *no change to the other has been found*. But may not such a change occur?

Whether or not the last suggestion ever proves to be true, it seems to me that from the data now collected one is entitled to discuss angiosperm endosperm formation from the viewpoint of experimental genetics.

The endosperm of the gymnosperms is essentially vegetative tissue of the female gametophyte. It results from continuous cell

formation originating with the germination of the megaspore, although fertilization occurs during the process. From the time of HOFMEISTER the morphological character of the endosperm of angiosperms was considered to be the same as that of the gymnosperms until the double fertilization was discovered. This fact gave rise to the idea that the angiosperm endosperm might be a sporophytic rather than a gametophytic structure, its nature being that of a monstrous embryo, or possibly that it is a composite tissue neither gametophytic nor sporophytic.

Most botanists, however, have held with STRASBURGER to the original idea that the endosperm is gametophytic. STRASBURGER concluded that the second fusion is not a true act of fertilization uniting the parental qualities and forming an embryo, but a vegetative fusion acting merely as a stimulus to growth. Miss SARGENT, however, believes that it is a degenerate embryo, the monstrous character being caused by the interference of the antipodal nucleus having a vegetative character and an indefinite and usually redundant number of chromosomes in the act.

The difficulty in the situation appears to be the obscurity of the phylogenetic history of the fusion of the two nuclei in the embryo sac and the subsequent fusion with the second male nucleus. The problem is further complicated by the irregularity of endosperm formation in various species. Although triple fusion appears to occur in the majority of angiosperms, the following important general variations have been noted. In addition to these general variations many minor deviations have been found (COULTER and CHAMBERLAIN **4**). (1) Vegetative endosperm formation may take place in a similar manner to that occurring in gymnosperms. This may occur without fertilization, or before or after fertilization. Usually the endosperm tissue is formed from the descendants of the antipodal cells, but the chalazal nucleus may degenerate and the endosperm be formed from the micropylar polar nucleus. (2) The polar nuclei may not fuse, but divide independently. (3) Fusion may include many cells.

Furthermore, endosperm formation may be initiated by free nuclear division, or the sac may be divided into two parts by a cell wall after the first division. Even when the latter phenomenon

occurs, endosperm tissue may be formed in both chambers, although usually division proceeds only in the micropylar chamber.

These general cytological data being given, how do the facts from pedigree cultures bear upon the problem?

Just how much weight should be given to data from only one species when discussing the morphological significance of the endosperm is questionable. But in maize it is evident that STRASBURGER'S distinction between vegetative and generative fertilization will not hold. Cytological work on other species does not bear out Miss SARGENT'S conception, since endosperms form quite regularly without the interference of the antipodal vegetative (?) nucleus. If the perfectly regular manner in which the above-mentioned endosperm characters of maize are transmitted is considered apart from other facts, there appears to be no escape from the conclusion that the endosperm is sporophytic in character. But there is another way of looking at the matter that makes the view of COULTER seem more probable.

COULTER (**3**) has concluded that conditions in the embryo sac favor fusions of any number or kind of free nuclei—an indefinite process without a necessary phylogeny that results in a growth which is practically gametophytic. It is not dependent upon a male nucleus, a polar nucleus, or even a reduction division.

The experimental evidence accords perfectly with this view. The superficial endosperm characters are indeed transmitted regularly when a male nucleus takes part in the fusion, but there is no reason for believing that the remaining maternal nuclei carry *all* the characters borne by the egg because *these* characters are the same in the nuclei concerned. The egg must usually have an organization somewhat different from that of the other maternal nuclei; although it is recognized that other nuclei sometimes function as eggs. It is likely that a differentiation has ensued which makes a particular nucleus an egg, and that it is not wholly a matter of position. The general belief in the vegetative character of the antipodal cells of the embryo sac is an admission that they have not received *all* the properties retained by other four cells. It is not very heretical, therefore, to assume that the cell that becomes the egg is different from its associates. Botanists hesitate to assume

the differentiation during ontogeny admitted by zoologists. They desire to believe that most plant cells can reproduce the whole plant. But this is a belief and not a fact, and until it becomes a fact it is well to recognize this plausible alternative in considering matters such as periclinal and sectorial chimeras as well as endosperms.

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BRIEFER ARTICLES

THE USE OF CELLOIDIN MEMBRANES FOR THE DEMONSTRATION OF OSMOSIS

(WITH THREE FIGURES)

Although the value of a celloidin film as an osmotic membrane is well known to botanists, little use of it has been made on account of the difficulties experienced in manipulation. A description of the method heretofore employed can be found in most textbooks on bacteriology. In this method, a celloidin solution is poured into a test tube or an Erlenmeyer flask and carefully rotated until a film which can be lifted out is formed on the inside of the tube, the sac thus formed being used as the membrane. Another method of preparing celloidin membranes, first described by BIGELOW and GEMBERLING,[1] is generally unknown to botanists, although considerable use has been made of it by chemists. This method consists in pouring a celloidin solution on a clean mercury surface from which the membrane thus formed is removed after the celloidin has sufficiently hardened. I have found that a three-inch Petri dish forms a very convenient container for the mercury. After carefully cleaning the surface of the mercury, enough 10 per cent celloidin solution is poured on it to cover an area 3–4 cm. in diameter. In 2–5 min. the ether and alcohol have evaporated sufficiently to allow the membrane to be lifted. It is important that the celloidin film be "ripe" before attempting to lift it; otherwise a stringy mass will be formed instead of a sheet. As soon as its edge appears scalloped and the surface shows small polygonal patches, the film can be raised. Its general appearance at the time it is "ripe" enough to be taken off is shown in fig. 1.

The best support for the osmotic membrane prepared as described above is the bulb of a thistle tube having a stem a half-inch in length, a thistle tube with a shortened stem being much more easily filled. It can be held in a test tube rack or in a small-necked bottle with the opening of the bulb uppermost and the celloidin membrane placed over its open end (fig. 2, *A*). The overlapping portion of the membrane should then be pressed to the sides of the flange of the bulb and tied securely with

[1] BIGELOW, S. L., and GEMBERLING, A., Collodion membranes. Jour. Am. Chem. Soc. **29**:1576–1599. 1907.

stout Manila twine. As the membrane dries it adheres tightly to the flange, and since it contracts somewhat on drying, it becomes stretched

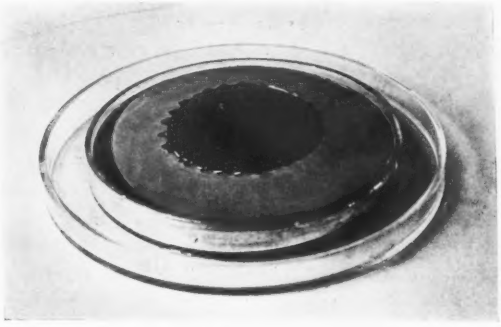

FIG. 1.—Celloidin film on a dish of mercury; the edges show the characteristic appearance at time when it is "ripe."

FIG. 2.—Different stages in the formation of celloidin membranes: *A*, membrane after being placed over the bulb of a thistle tube; *B*, showing how the film adheres to the flange; *C*, after tying the membrane on the thistle tube; *D*, a double membrane.

as taut as the head of a drum (fig. 2, *B*). The ends of the twine are then cut off and the part of the film which extends more than a quarter of an

inch beyond the twine should be trimmed off with a sharp knife (fig. 2, *C*). This trimmed membrane after further drying for an hour should then be kept in a dish of water until needed for use. MATHEWS[2] dried his membranes over a steam coil for several days, but found that perfectly dry membranes did not allow the solute to pass through very rapidly.

As considerable variation was found in membranes made in the same manner and at the same time, various attempts were made to reinforce them and make their tensile strength more uniform. The method used for testing a membrane consisted in suspending the osmometer and then pouring mercury into the tube until the membrane broke, noting the height of the column of mercury in the tube at that time. Usually there was not a rupture of the entire membrane, but a small leak in one place, and that generally near the flange of the bulb. The different methods used in making the membranes, and their tensile strength, as indicated by the height of the mercury column, are shown in the table given below.

TABLE SHOWING THE RELATIVE STRENGTH OF VARIOUS MEMBRANES

The figures refer to the height (in cm.) of the column of mercury when the membrane broke.

Method of formation	1st	2d	3d	4th	5th	Average
Not tied; put in water 5 min. after being made	13.5	8.0	14.0	.....	.....	11.83
Not tied; air dried one hour, then put in water	10.5	4.5	11.5	16.0	.....	10.39
Membrane reinforced with bar netting; not tied	17.0	12.5	19.0	.....	.....	16.17
Not tied; hardened in chloroform 48 hours	15.0	12.5	15.0	.....	.....	14.17
Double membrane; not tied	73.0	29.0	73.5	54.0	64.0	58.70
Single membrane, tied; air dried 24 hours	9.0	59.0	11.0	8.5	41.0	25.70
Same, then soaked 24 hours in water	27.0	8.0	62.0	52.0	22.0	34.20
Double membrane, tied and dried in air an hour	94.0	36.0	238.0	230.0*	215.0*	163.40

* No break in the membrane occurred when the height of the mercury column had reached this point.

As the above tests show, the double membranes are by far the strongest. These are produced by making a second single membrane and superimposing it over one made in the manner described above (fig. 2, *D*). There may be one or two bubbles of air between the two

[2] MATHEWS, J. H., Osmotic experiments with collodion membranes. Jour. Phys. Chem. **29**: 281–291. 1910.

membranes, but these do not seem to interfere with the work or the strength of the membrane.

In three days an osmometer made in this manner and filled with a 25 per cent cane sugar solution, under ordinary laboratory conditions, will send up a column of liquid 130–197 cm. high. When similar osmometers are filled with a 10 per cent sodium chloride solution, the liquid will rise 138–172 cm. in the same length of time and under the same conditions. At the end of this time the water column begins to sink slowly.

Attempts were made to render membranes semipermeable by using tannic acid and copper ferrocyanide. Osmometers having membranes prepared as described above were filled with a 5 per cent tannic acid solution and then set in dishes containing the same solution. Enough melted 10 per cent gelatin solution to form a film was poured in the bottom of other osmometers, allowed to harden, and then treated with the 5 per cent tannic acid as before. After standing in the tannic acid solution for 48 hours, the osmometers were placed in water to soak out the excess of tannic acid. The acid leeched out for a long time afterward. In some cases the leeching continued for as long as three weeks, even after several changes of water. On account of the difficulty of freeing the membranes of the excess of tannic acid, no further experiments were made with this substance.

For the formation of the copper ferrocyanide membranes, M/20 solutions of copper sulphate and potassium nitrate were used. Osmometers were filled with the copper sulphate solution and immersed in the potassium ferrocyanide solution, while others containing potassium ferrocyanide were placed in the copper sulphate solution (fig. 3). The latter method proved the better, as PFEFFER[3] found in his experiments with porous clay cups. By the end of the third day the celloidin film becomes impregnated with the copper ferrocyanide. There may be some exosmosis of the potassium ferrocyanide and a consequent formation of copper ferrocyanide on the outside of the membrane, but this is easily washed off with water. The precipitation membrane is not formed uniformly throughout the celloidin, but has a mottled appearance at first. Later the membrane becomes uniform in color and texture.

These membranes need not be used at once, but should be used within a month. It was found by BIGELOW and GEMBERLING that for celloidin membranes standing in water for three months the permeability is considerably decreased. The rapidity of the passage of the liquid

[3] PFEFFER, W., Osmotische Untersuchungen. Leipzig. 1877.

through the membranes and its consequent rise in the osmometer varies considerably, the osmometers with the smaller, dryer membranes showing a much slower rise. Membranes with a large osmotic surface, for example 5 cm. in diameter, will allow the liquid to rise quite rapidly.

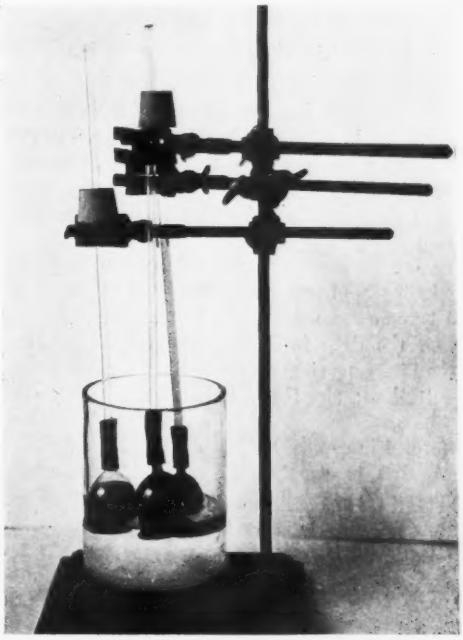

FIG. 3.—Osmometers filled with potassium ferrocyanide solution and immersed in a copper sulphate solution; the precipitation membrane is being formed inside of the celloidin film.

When an osmometer prepared in this manner is set up, water will rise to a much greater height than in those having untreated celloidin membranes. In one experiment with this type of membrane the water rose 8 meters.

I am indebted to Professor J. B. OVERTON for advice and criticism during the progress of this work, for which I take this opportunity of expressing my appreciation.—GILBERT MORGAN SMITH, *University of Wisconsin, Madison, Wisconsin.*

CURRENT LITERATURE

BOOK REVIEWS

Rhythm, periodicity, and zonation

KÜSTER's booklet[1] on zone formation in colloidal media contributes little if anything to botany as such, but is well worth while in presenting a point of view held by a number of continental workers. The first chapter (pp. 23) deals with equidistant zones, the second (pp. 41) with breaks, dislocations, etc., in the zones, the third (pp. 32) with excentric ring systems and polycentric diffusion fields, and the fourth (pp. 7) with zoological considerations and discussion. His point of departure is the Liesegang rings. The concentric rings are produced in a few hours after placing a droplet of 80 per cent $AgNO_3$ on 5–10 per cent gelatin plate bearing 0.1 per cent $K_2Cr_2O_7$. The rings consist of Ag_2CrO_4 and become more definite and more distant from each other as the distance from the drop increases. The author mentions OSTWALD'S explanation of the Liesegang rings on the basis of zones of stable, metastable, and labile concentrations; also the fact that the explanation has been questioned.

The bands formed in capillary tubes show definite rhythm and polarity. Aside from the Ag_2CrO_4 bands, there are others caused by impurities in the gelatin which KÜSTER terms small rhythms, in contrast to the great rhythms of Ag_2CrO_4. Many conditions modify the patterns given by the precipitate of Ag_2CrO_4: contact with the dish in contrast to free gelatin, presence of foreign bodies in the gelatin, tensions and pressures, and others. In fact, patterns can be produced that resemble closely all the various patterns appearing in mottled leaves, in the arrangement of vascular elements of plants, and in markings of tracheae.

The writer emphasizes the fact that the pattern differentiation of Ag_2CrO_4 precipitate shown in the gelatin plate is a self-differentiation occurring under constant environmental conditions. This is taken to show that similar periodic or rhythmic structural changes in the cell may be independent of rhythm in the environment, a matter of self-differentiation. The work also indicates the possibility of the independence of such differentiation from vitalistic peculiarities. The fact that a simple diffusion process in gelatin gives complex polarized precipitation patterns leads the author to conclude that perhaps the complex patterns in the organism may likewise be referred to a simple diffusion

[1] KÜSTER, ERNST, Über Zonenbildung in kolloidalen Medien. Beiträge zur entwicklungsmechanischen Anatomie des Pflanzen (erstes Heftes). pp. x+111. *figs.* 53. Jena: Gustav Fischer. 1913.

process in a colloidal matrix, rather than explained by appeal to complex regulation processes. While this work deals in the main with analogies in morphological features, the writer emphasizes the fact that dynamic activities in the non-living often show rhythmic action determined by internal characters of the system rather than change of the environment. He cites, along with several other examples, the periodic elimination of oxygen when a clean mercury surface is covered with a neutral or slightly alkaline solution of hydrogen peroxide. In his later work the writer will undoubtedly turn more to the dynamic.

Rhythm in plant activity is held by many to be generally related to environmental rhythm. Others feel that rhythm is a necessity of the very nature of protoplasmic activity; activity must be followed by rest. An examination of the facts that KÜSTER offers shows the possibility of rhythms in the organism independent of environmental rhythm, and as well independent of the inscrutable features of protoplasm. In short, internally determined rhythms may be matters of relatively simple chemical and physical laws.—WILLIAM CROCKER.

Makers of British botany

Under this title, Professor OLIVER has edited a most interesting book.[2] The book grew out of a series of ten lectures delivered by various botanists at the University College of London in 1911. These lectures have been supplemented by six other chapters, so that the work might be more fully representative.

The botanists included and those who prepared the sketches are as follows: ROBERT MORISON (1620–1683) and JOHN RAY (1627–1705), by S. H. VINES; NEHEMIAH GREW (1641–1712), by Mrs. ARBER; STEPHEN HALES (1677–1761), by FRANCIS DARWIN; JOHN HILL (1716–1775), by T. G. HILL; ROBERT BROWN (1773–1858), by J. B. FARMER; Sir WILLIAM HOOKER (1785–1865), and Sir J. D. HOOKER (1817–1911), by F. O. BOWER; J. S. HENSLOW (1796–1861), by GEORGE HENSLOW; JOHN LINDLEY (1799–1865), by FREDERICK KEEBLE; WILLIAM GRIFFITH (1810–1845), by W. H. LANG; ARTHUR HENFREY (1819–1859), by F. W. OLIVER; WILLIAM HENRY HARVEY (1811–1866), by W. LLOYD PRAEGER; MILES BERKELY (1803–1889), by GEORGE MASSEE; Sir JOSEPH GILBERT (1817–1901), by W. B. BOTTOMLEY; W. C. WILLIAMSON (1816–1895), by D. H. SCOTT; HARRY MARSHALL WARD (1854–1905), by W. THISELTON-DYER; The Edinburgh professors (1670–1887), by I. BAYLEY BALFOUR.

It is impossible to review such a book in a brief space, but botanists will be glad to know where biographies of these botanists may be obtained. Each one stood for some phase of botany and contributed his share to its history. —J. M. C.

[2] OLIVER, F. W., Makers of British botany, a collection of biographies by living botanists. 8vo. pp. 332. *pls. 26*. Cambridge: University Press. 1912. 9*s*.

Protein metabolism

CATHCART[3] has added a volume on metabolism of proteins to the series of monographs on biochemistry being issued by the publishers cited. There are nine chapters under the following headings: digestion and absorption of proteins, protein regeneration, feeding experiments with abiuret products of digestion, deaminization, influence of food on the composition of the tissues, protein requirements, theories of protein metabolism, starvation, work.

The author says: "The present monograph does not pretend to cover the whole literature of protein metabolism; it consists rather of the discussion of the more important results published during the last decade." Yet the bibliography includes 430 citations. This indicates how extensive is the work done on the protein metabolism of mammals alone as compared with the scattering contributions to protein metabolism in plants. This little volume makes readily available to plant physiologists the status of our knowledge of this subject in mammals. It brings one to the realization of some of the striking similarities between protein metabolism in plants and in mammals, as for instance the power of deaminization. One also comes to appreciate some of the differences appearing in the two groups of organisms, as is shown in the power of synthesizing amino acids.—WILLIAM CROCKER.

Herbals

Mrs. ARBER[4] has brought to the knowledge of botanists some very interesting information concerning the early history of botany. She has given a general sketch of the evolution of the printed herbal from 1470 to 1670 from a botanical and also an artistic standpoint. Many of these herbals are extremely rare, and Mrs. ARBER has done good service in making them more real to botanists. Of course the text is full of interest, but the numerous illustrations introduce one even more vividly into the botanical atmosphere of three or four centuries ago.

A list of the nine chapter titles will give a better conception of the contents of the volume: (i) "The early history of botany"; (ii) "The earliest printed herbals"; (iii) "The early history of the herbal in England"; (iv) "The botanical renaissance of the sixteenth and seventeenth centuries"; (v) "The evolution of the art of plant description"; (vi) "The evolution of plant classification"; (vii) "The evolution of the art of botanical illustration"; (viii) "The doctrine of signatures, and astrological botany"; (ix) "Conclusions." —J. M. C.

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[3] CATHCART, E. P., The physiology of protein metabolism. pp. viii+142. New York: Longmans, Green & Co. 1912.

[4] ARBER, AGNES, Herbals, their origin and evolution, a chapter in the history of botany (1470–1670). 8vo. xviii+253. *pls. 22. figs. 113.* Cambridge: University Press. 1912.

MINOR NOTICES

The fresh-water flora of Germany, Austria, and Switzerland.—PASCHER[5] has begun the publication of a series of brochures dealing with the fresh-water flora of Germany, Austria, and Switzerland, assisted by numerous specialists. The plan includes 16 parts, 4 of which have just appeared: no. 2 (pp. 192. *figs. 398*), Flagellatae 2, by A. PASCHER (Chrysomonadinae, Crytomonadinae, and Chloromonadineae) and E. LEMMERMANN (Eugleninae); no. 3 (pp. 66. *figs. 69. M* 1.80), Dinoflagellatae (Flagellatae 3), by A. J. SCHILLING; no. 9 (pp. 51. *figs. 89. M* 1.80), Zygnemales, by O. BORGE and A. PASCHER; no. 10 (pp. 192. *figs. 398. M* 4), Bacillariales (Diatomeae), by H. v. SCHÖNFELDT. The numerous illustrations and analytical keys should make the recognition of forms comparatively easy.—J. M. C.

William Russell Dudley.—Leland Stanford Junior University has published a "Dudley Memorial Volume," containing a paper by the late Professor DUDLEY and appreciations and contributions by friends and colleagues. The list of scientific papers is as follows: "The vitality of *Sequoia gigantea*," by W. R. DUDLEY; "The morphology and systematic position of *Calycularia radiculosa*," by D. H. CAMPBELL; "Studies of irritability in plants. III. The formative influence of light," by G. J. PEIRCE; "The gymnosperms growing on the grounds of Stanford University," by LEROY ABRAMS; "The Synchytria in the vicinity of Stanford University," by JAMES MCMURPHY; "The law of geminate species," by D. S. JORDAN; "Some relations between salt plants and salt spots," by W. A. CANNON; "North American species of the genus *Amygdalus*," by W. F. WIGHT.—J. M. C.

NOTES FOR STUDENTS

Cultures of the Uredineae.—The publications of 1912 on cultural work with the plant rusts show an increasing tendency in all parts of the world to clear up by systematic efforts rather than by sporadic cultures the problems of biological relationships in this group of parasitic fungi. In the United States, ARTHUR, who for many years has been prominently associated with this field of research, reports[6] six species which either have been shown to be autoecious or have been connected with their antithetic generation for the first time. These are as follows:[7] *Puccinia Lygodesmiae* Ellis et Ev. from *Lygodesmia juncea* (Pursh) D. Don produced teleutospores on the same host without the intercalation of pycnidia or other spore forms. Aecidiospores of *Aecidium monoicum* Peck from *Arabis* sp. produced uredinia and telia on

[5] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Osterreichs, und der Schweiz. Parts 2, 3, 9, and 10. Jena: Gustav Fischer. 1913.

[6] ARTHUR, J. C., Cultures of Uredineae in 1911. Mycologia **4**:49–65. 1912.

[7] Unless otherwise stated, teleutosporic material was used in making the infections and the teleutosporic host is given first.

Trisetum subspicatum (L.) Beauv. and *T. majus* (Vasey) Rydb.; the species is described as *Puccinia monoica* (Peck) Arthur. *Gymnosporangium Nelsoni* Arth. (*G. durum* Kern) from *Juniperus utahensis* (Engelm.) Lemmon produced only pycnidia on *Amelanchier vulgaris* Moench. *Gymnosporangium effusum* Kern from *Juniperus virginiana* L. produced only pycnidia (*Roestelia transformans* Ellis?) on *Aronia arbutifolia* (L.) Ell. *Gymnosporangium gracilens* (Peck) Kern et Bethol. from *Juniperus monosperma* (Engelm.) Sarg. produced pycnidia and aecidia on *Philadelphus coronarius* L. In addition to these, cultures of 15 species are reported as confirming previous work.

FRASER,[8] who has been investigating the rusts in Canada, especially those inhabiting conifers, reports the following species whose relations have been worked out for the first time. *Necium Farlowii* Arthur from *Tsuga canadensis* sown on the same host produced only teleutospores but no pycnidia. *Melampsoropsis* (*Chrysomyxa*) *Pyrolae* (DC.) Arth. from *Pyrola americana* Sweet and *P. elliptica* Nutt. produced pycnidia and aecidia (*Peridermium conorum-Piceae* [Schw.] Arth.) on cones of *Picea mariana* (Mill.) B.S.P. and *P. canadensis* (Mill.) B.S.P. *Pucciniastrum minimum* (Schw.) Arth. from *Rhodora canadense* (L.) B.S.P. produced aecidia (*Peridermium Peckii* Thüm.) on leaves and cones of *Tsuga canadensis* (L.) Carr. *Uromyces Spartinae* Farl. from *Spartina Michauxiana* Hitch. infected *Arenaria lateriflora* L., but failed to infect *Spergularia canadensis* (Pers.) Don, while the same rust from *Spartina patens* (Ait.) Muhl. and *S. glabra* var. *alterniflora* (Loisel) Merr. infected *Spergularia canadensis*, but failed to infect *Arenaria lateriflora* L. *Melampsora arctica* Rostr. from *Salix discolor* Muhl. produced pycnidia and aecidia (*Caeoma* sp.) on *Abies balsamea* (L.) Mill. *Melampsora* (*Medusae* Thüm?) from *Populus grandidentata* Michx. produced pycnidia and aecidia (*Caeoma Abietis canadensis* Farl.) on *Tsuga canadensis* (L.) Carr., and in one case pycnidia on *Larix laricina*. Cultures confirming former work are reported for 12 species.

Of great interest is the discovery of the relation between certain fern rusts and the aecidial forms known as *Peridermium balsameum* Peck on *Abies balsamea* (L.) Mill. This relationship, suggested in this paper, is confirmed in a later note,[9] in which it is stated that *Uredinopsis Osmundae* Magnus, *U. Struthiopteridis* Störmer, *U. Phegopteridis* Arthur, *U. mirabilis* (Peck) Arthur, and *U. Aikinsonii* Magnus have as their aecidial stages forms of *Peridermium balsameum* Peck on *Abies balsamea*. A complete account is promised.

HEDGCOCK[10] succeeded in establishing the relation between *Peridermium filamentosum* Peck and the *Cronartium* on species of *Castilleja*. The aecidi-

[8] FRASER, W. P., Cultures of heteroecious rusts. Mycologia **4**:175–193. 1912.

[9] ———, Note on the life histories of the fern rusts of the genus *Uredinopsis*. Science N.S. **36**:595. 1912.

[10] HEDGCOCK, G. C., The *Cronartium* associated with *Peridermium filamentosum* Peck. Phytopathology **2**:176–177. 1912.

ospores were sown on two unidentified species of *Castilleja* and produced uredinia of a species of *Cronartium* for which the name *C. filamentosum* is proposed.

In Europe several papers on cultural work with rusts have appeared. Prominent among them is a paper by KLEBAHN,[11] who reports the results of cultural experiments carried out from 1907 to 1911. Many of the results are new, while others confirm or supplement the work previously published by the author or other investigators. Unfortunately, the presentation is not sufficiently clear and concise to enable one to distinguish those which are new from those which confirm other work. In this respect the reports of ARTHUR, and likewise that of FRASER, might well serve as models. The main results given in the paper are summarized here. *Uromyces Pisi* (Pers.) DeBary, sown on the young subterranean shoots of *Euphorbia Cyparissias* L. in the spring of 1906, produced the usual deformations with pycnidia and aecidia in the spring of 1907, thus confirming the work of JORDI. Aecidiospores from this culture infected *Pisum sativum* L. In another experiment *Lathyrus vernus* Bernh. was infected by aecidiospores from *E. Cyparissias* collected in another locality. This result suggests that several forms of *Uromyces* have their aecidia on that host. *Uromyces lineolatus* (Desm.) Schroet. from *Scirpus maritimus* L. infected *Berula angustifolia* Koch, *Oenanthe aquatica* Lamarck, and *Hippuris vulgaris* L. Aecidiospores of *Puccinia argentata* (Schultz) Winter from *Adoxa Moschatellina* L. infected leaves of *Impatiens nolitangere* L. and the subterranean shoots but not the developing leaves of *Adoxa*. It appears, therefore, that the infection of *Adoxa* takes place only in the subterranean parts. A form of *Puccinia* on *Carex crinita* Lam. infected *Ribes aureum* Pursh, *R. alpinum* L., *R. Grossularia* L., and *R. Cynosbati* L. This type, which was described by ARTHUR as *P. albiperidia*, the author does not regard as distinct from *P. Ribesii-Caricis* Kleb. Another form which had not been tested, and which was collected on *Carex teretiuscula* Gooden., produced aecidia on *Ribes rubrum* L., *R. alpinum* L., and also on *Urtica dioica* L. This was therefore a mixture of *P. Ribesii-Caricis* and *P. Urticae-Caricis*. A *Puccinia* from *Carex ligerica* Gay produced aecidia on *Taraxacum officinale* Web. This rust, therefore, belongs to *Puccinia silvatica* Schröter, which is a collective species whose forms have not been separated. *Puccinia Polygoni amphibii* Pers. on *Polygonum amphibium* L. infected *Geranium Phaeum* L., *G. pratense* L., and *G. sanguineum* L., while *Puccinia Polygoni* Alb. et Schwein. from *Polygonum Convolvulus* infected *Geranium molle* L., but none of the others.

The existence of specialized forms of *Puccinia Smilacearum-Digraphidis* Kleb. was confirmed in the following manner. Aecidiospores from *Paris quadrifolia* L. produced an abundance of teleutospores on *Phalaris arundinacea*. The teleutospores from this culture produced aecidia freely on *Paris quadri-*

[11] KLEBAHN, H., Kulturversuche mit Rostpilzen. Zeitschr. Pflanzenkrankh. **22**:321–350. 1912.

folia and *Polygonatum multiflorum*, but sparsely on *Convallaria majalis* L. and *Majanthemum bifolium* Schmidt. Teleutospores of the same species from another region infected *Convallaria* freely, but produced only yellow spots on the other three liliaceous plants. Aecidiospores of *Puccinia Symphyti-Bromorum* F. Müller from *Symphytum officinale* L. infected *Bromus inermis* Leyss., *B. erectus* Huds., *B. rigidus* Roth, and *B. mollis* L., thus confirming the relation between this aecidium and the brome rusts. Out of 15 species of plants on which *Puccinia persistans* Plowright from *Agropyrum repens* Beauv. was sown, only *Thalictrum flavum* L. was infected.

With the crown rusts of grasses the following cultures were made. (1) Aecidiospores obtained by sowing *Puccinia coronata* f. *agrostidis* Erikss. from *Agrostis vulgaris* With. on *Rhamnus Frangula* (*Frangula Alnus* Mill.) infected only *Agrostis alba* L. of the 8 grasses used. Since plants of *Calamagrostis* were not infected, this result is further evidence for the distinctness of the forms of crown rusts on *Agrostis* and on *Calamagrostis*. (2) Aecidiospores obtained by sowing *Puccinia coronifera* f. *Lolii* Erikss. on *Rhamnus cathartica* L. infected *Lolium perenne* L., *L. temulentum* L., *Festuca elatior* L., and *Holcus lanatus* L. This confirms the results of other experiments that the crown rusts known as the forms *Lolii* and *Festucae* are not well differentiated. (3) Aecidiospores obtained by sowing *Puccinia coronifera* f. *Holci* Kleb. from *Holcus lanatus* L. on *Rhamnus cathartica* infected both *Holcus lanatus* and *Lolium perenne*, showing that the forms *Holci* and *Lolii* are likewise not sharply differentiated. (4) A crown rust on *Arrhenatherum elatius* Mert. et Koch was found to produce aecidia on *Rhamnus cathartica*, and thereby proved to belong to *Puccinia coronifera* Kleb. Since the aecidiospores infected *Arrhenatherum elatius* but none of the other grasses subject to the attacks of crown rusts, this is considered by the author as a special form, *Arrhenatheri*.

Cultures with *Phragmidium Rubi* (Pers.) Winter, *P. violaceum* (Schultz) Winter, and *Kuehneola albida* (Kühn) Magnus on some 26 species of *Rubus* showed that no specialized races exist within the species in these rusts; the species, however, show differences in their behavior toward different species of *Rubus*. *Phragmidium Rubi* infected *Rubus caesius* L. and the species of the CORYLIFOLII group, but only rarely the species of other groups. *P. violaceum* infected most of the other species but not *R. caesius*, and only rarely the members of the CORYLIFOLLII group. *Kuehneola* infected nearly all the species. Sowing of aecidiospores of *Melampsora vernalis* Mieszl. from *Saxifraga granulata* L. on the same host confirmed the experiments of PLOWRIGHT and of DIETEL according to which this rust is autoecious. A number of cultures did not give positive results. Sowings of uredospores of *Uromyces Alchemillae* (Pers.) Winter on *Alchemilla* gave no infection, although successful infections have been previously reported both by the author and by FISCHER. The life history of this fungus, whose mycelium is perennial in the rootstocks of *Alchemilla*, has not been sufficiently cleared up. *Puccinia Tanaceti* DC. from *Tanacetum*

vulgare L. failed to infect that host. *P. Pringsheimiana* Kleb. grown on *Carex acuta* L. failed to infect *Ribes nigrum* L., although *R. Grossularia* L. was freely infected. An attempt to confirm LIRO's observation that *Pedicularis palustris* L. is the teleutospore host of *Peridermium Pini* Willd. (Kleb) was not successful. Sowings of aecidiospores on a number of other possible hosts resulted in failure, so that the teleutospore host of this important rust is still unknown. Aecidiospores of *Aecidium Circaeae* Ces. from *Circaea lutetiana* L. failed to infect *Brachypodium silvaticum* R. Sch., which was regarded as a possible alternate host for this fungus.

In conclusion, the author adds some observations on the wintering of uredospores of grain and other rusts. The uredospores of *Melampsoridium betulinum* (Pers.) Kleb., *Melampsora Larici Tremulae* Kleb., *Thecopsora Vaccinii* (Alb. et Schw.) Winter, and *Kuehneola albida* (Kühn) Magnus were incapable of surviving the winter. Some observations on an unknown species of *Melampsora* on *Populus alba* L. indicate that the mycelium of this fungus persists in the buds and produces uredinia on the unfolding leaves. All attempts to infect grasses with teleutospores of *Puccinia graminis* failed. To account for the appearance of the grain rusts in spring it has been suggested that the fungus is carried over with the seed. To test this hypothesis further, the author planted a number of plots with seed from plants badly infected with rust, but the young plants were free from rust in every case.

FISCHER publishes two papers on the specialization of certain rusts. The first[12] deals with specialization of forms within the collective species *Puccinia Saxifragae* Schlecht. inhabiting various European species of *Saxifraga*. DIETEL and H. and P. SYDOW have shown that the forms associated under that name can be separated into a number of species morphologically well marked. The possibility that within these species there might exist physiological races not morphologically distinguishable led FISCHER to investigate the *Puccinia* on *Saxifraga stellaris* in Norway. Sowings of the teleutospores were made on *S. stellaris*, *S. rotundifolia*, *S. androsacea*, *S. nivalis*, and *Aizoon longifolia*. Only *S. stellaris* was infected. In the course of these experiments, FISCHER observed that the newly formed teleutospores germinated immediately and continued during the entire summer to infect the leaves of the plants on which they were borne. The teleutospores of this species, therefore, act indifferently like those of a micro-*Puccinia* or of a lepto-*Puccinia*. A differentiation of the teleutospores into persistent and caducous types, which occurs in other species with teleutospores of similar behavior, does not occur in this species.

In the second paper[13] FISCHER gives further results of his investigations on the life histories of forms of *Uromyces caryophyllinus* (Schrank) Winter.

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[12] FISCHER, ED., Beiträge zur Biologie der Uredineen. 2. Zur Biologie von *Puccinia Saxifragae* Schlecht. Mycol. Centralbl. **1**:277–284. 1912.

[13] ———, Beiträge zur Biologie der Uredineen. 3. Die Specialization des *Uromyces caryophyllinus* (Schrank) Winter. Mycol. Centralbl. **1**:307–313. 1912.

Formerly FISCHER[14] had shown that the aecidiospores of the aecidium on *Euphorbia Gerardiana* collected in a district of Switzerland infected *Saponaria ocymoides*, but not other members of the pink family. Later, in a preliminary paper,[15] he described the successful infection of *Tunica prolifera* by aecidiospores from the same *Euphorbia* collected in another region (near Heidelberg). The existence of specialized forms within the species *Uromyces caryophyllinus* suggested by this experiment was confirmed by the experiments reported in the present paper. In this series of cultures the aecidiospores from *Euphorbia Gerardiana* collected near Heidelberg were sown on plants representing 10 species of the Caryophyllaceae, but only *Tunica prolifera* was infected. In single instances one uredo pustule was observed on *Saponaria ocymoides* and one on *Tunica Saxifraga*. A repetition of sowings of aecidiospores, collected in the region from which the material first used to infect *Saponaria ocymoides* was obtained, resulted in a doubtful infection of *Saponaria*, but a fairly abundant infection of *Tunica prolifera*. It is probable, therefore, that this material consisted mostly of the *Tunica*-form. These experiments show that *Uromyces caryophyllinus* consists of at least two biologic forms, one *Tunica prolifera*, rarely infecting *Saponaria ocymoides*, and one on *S. ocymoides*, whose relations to *Tunica prolifera* are not fully known.

SCHNEIDER[16] reports the following results of cultures of rusts infecting liliaceous plants. *Uromyces Scillarum* (Grev.) Winter, which occurs on various species of *Muscari* and *Scilla*, infected only *Muscari racemosum*, the species from which the material had been obtained. The teleutospores were found to germinate either immediately or after a period of dormancy. *Puccinia Schroeteri* Passerini from *Narcissus radiiflorus* infected also *N. pseudonarcissus*. *Puccinia Allii* (DC.) Rudolphi is reported by SYDOW as occurring on 27 species of *Allium*. Teleutospores from *Allium sphaerocephalum* produced uredinia on that species and also on *A. sativum*, *A. hymenorrhizum*, *A. oleraceum*, and *A. fistulosum*. In one case pycnidia and aecidia appeared on *A. sativum*. Uredospores of *Puccinia Porri* (Schw.) Winter from *Allium Schoenoprasum* infected *A. Schoenoprasum*, and to a less degree *A. ampeloprasum*, *A. sphaerocephalum*, *A. strictum*, *A. montanum*, *A. fistulosum*, *A. oleraceum*, and *A. hymenorrhizum*. In one case, a sowing of teleutospores from *A. Schoenoprasum* on plants of that species resulted in the production of aecidia. This fact is of interest, since TRANZCHELL,[17] as a result of experiments with the same form, found that no aecidia were formed, whence he concluded that this was a true hemi-*Puccinia*. The existence of hemi-forms is therefore still in doubt.

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[14] Rev. BOT. GAZ. **53**:79. 1912.

[15] FISCHER, ED., Über die Specialization des *Uromyces caryophyllinus* (Schrank) Winter. Mycol. Centralbl. **1**:1, 2. 1912.

[16] SCHNEIDER, W., Zur Biologie der Liliaceen bewohnenden Uredineen. Centralbl. Bakt. **32**:452, 453. 1912.

[17] Rev. BOT. GAZ. **53**:80. 1912.

TREBOUX,[18] working at Nowotscherkassk in southeastern Russia, reports the results of a number of infection experiments carried out in that region. Since the literature on the subject was not available to him, he was unable to consider the work of former investigators. The following is a list of the successful infections. Aecidiospores from *Ranunculus illyricus* L. infected *Festuca ovina* L. (*Uromyces Festucae* Syd.). Aecidiospores from *Sium lancifolium* MB. infected *Scirpus maritimus* L. (*Uromyces lineolatus* [Desm.] Schroet.). Aecidiospores from *Euphorbia virgata* W.K. infected *Astragalus hypoglottis* L. (*Uromyces Astragali* [Opiz.] Sacc.). In former experiments, uredospores from *Astragalus virgatus* Pall. infected *A. cicer* L., *A. glycyphyllus* Pall., *A. ponticus* Pall., *A. cruciatus* Link., *A. hamaeus* L., *A. placatus* Lam., *A. tianchanicus* Bunge., *A. viciaefolius* DC., and *A. virgatus* Pall. Apparently there is no specialization of forms within this species of *Uromyces*. Aecidiospores from *Euphorbia virgata* W.K. and *E. Gerardiana* Jacq. infected *Caragana frutescens* DC. (*Uromyces Genistae-tinctoriae* [Pers.] Fuckel). Other leguminous plants, including *Astragalus* and *Medicago*, were not infected either by aecidiospores or by uredospores. Aecidiospores from another aecidium on *Euphorbia virgata* infected *Medicago falcata* (*Uromyces striatus* Schroet.), but not *Astragalus*, *Caragana*, and others. Aecidiospores from *Cichorium Intybus* L. infected *Juncus Gerardi* Lois (*Puccinia Junci* [Strauss] Winter). A sowing of aecidiospores from *Taraxacum serotinum* W.K. produced only uredospores, probably those of *Puccinia silvatica* Schroet., on *Carex stenophylla* Wahlenb. Uredospores of *Puccinia Cesatii* Schroet. on *Andropogon Ischaemum* L., which had persisted during an unusually severe winter, were capable of germinating and infecting that host in spring. Apparently this rust lives through the winter in that region by means of uredospores which have thick walls. Teleutospores of *Puccinia Stipae* (Opiz.) Arthur from *Stipa Lessingiana* Trin. infected *Salvia aethiopis* L., *S. nutans* L., *S. silvestris* L., *Thymus serpyllum* L., and *Ajuga chia* Schreb.

STRELIN,[19] in a series of cultures, shows the relation between the primary uredinia (*Uredo Muelleri* Schroet.) and secondary uredinia and telia of *Kuhneola albida* (Kühn) Magnus, confirming the work of JACKY showing that these spore forms all belong to the same rust. STRELIN also shows that the fungus persists through the winter by means of the spores of the primary uredinia which are formed in July and August. The primary uredospores can infect the following spring only the old leaves which have persisted through the winter.—H. HASSELBRING.

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[18] TREBOUX, O., Infektionsversuche mit parasitischen Pilzen. I. Ann. Myc. **10**:73–76. 1912.

[19] STRELIN, S., Beiträge zur Biologie und Morphologie der *Kuehneola albida* (Kühn) Magn. und *Uredo Muelleri* Schroet. Mycol. Centralbl. **1**:92–96, 131–137. 1912.

Cecidology.—Among the most important of the foreign contributions is a paper by KARNY,[20] in which the author describes a large number of gall-making *Thysaneura*, including a number of new species. However, the emphasis is placed upon the animal rather than the plant side of the subject.

A most excellent paper by HOUARD[21] on the galls of French West Africa gives descriptions of 51 new species of gall-makers on 29 species of host plants. The author describes both insect and cecidia and in most cases gives illustrations of the cecidia. The grouping is with reference to the plants, a practice which is apparently growing in favor with the students of cecidology.

Root nodules are the subject of two papers. Miss SPRATT[22] discusses the root nodules on the Podocarpineae which are caused by *Pseudomonas radicicola*. These nodules are modified lateral roots. The organism penetrates the root-hairs and then the cortex, and the stimulus is in the meristimatic tissue, but there is no differentiation of the meristimatic zone in the cortical tissue such as is found in the nodules of other non-legumes. The organism produces a zooglea in the cells which stimulates the nuclei of the host and causes them to divide amitotically. In the spring the cells immediately below the endodermis at the apex of the nodular stele become meristematic and produce new cortical cells in the interior of the old nodes. The other paper on root nodules is by BOTTOMLEY,[23] who finds the nodules on the *Myrica Gale* are also modified lateral roots. These nodules are also caused by *P. radicicola* and each mature nodule shows four zones: (*a*) the apical meristem, (*b*) the infected thread area, (*c*) the bacterial zone, (*d*) the basal zone in which the cells contain oil drops. The bacteria eventually disappear and the basal zone is replaced by the other zones.

A very important contribution to our knowledge of American cecidology is BESSEY'S[24] study of the nematode root knots. This disease is caused by *Heterodera radicicola* (Greef) Mull., and was probably indigenous in some tropical region of the Old World from which it has been distributed throughout the tropics and a considerable part of the temperate zones. Records show that about 480 species and varieties of plants, including nearly all the larger families, are subject to this disease. The life cycle is four weeks or more, dependent on the temperature of the soil. The author gives a good discussion

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[20] KARNY, K. Gallenbewohnende Thysanopteren aus Java. Marcellia **11**:115–169. 1912.

[21] HOUARD, C., Les galles de l'Afrique occidentale française (*V. Cecidies nouvelles*). Marcellia **11**:176–209. 1912.

[22] SPRATT, ETHEL ROSE, The formation and physiological significance of root nodules in the Podocarpineae. Ann. Botany **26**:803–813. 1912.

[23] BOTTOMLEY, W. B., The root nodules of *Myrica Gale*. Ann. Botany **26**:111–117. 1912.

[24] BESSEY, E. A., The root knot and its control. U.S. Bur. Pl. Industry, Bull. 217. 1911.

of the life history of the organism, methods of distribution, and methods of control.

The potato eelworm is the subject of a brief paper by ESSIG.[25] He describes the disease and the organism and suggests treatments. The disease is of such great importance that California has established a quarantine against potatoes from infected districts.

A recent importation from Europe is reported by CRAWFORD.[26] It is *Triozoa alacris* Flor., which causes a rolling and distorting of the leaves of *Laurus nobilis* and other species of *Laurus*.

The olive knot is the subject of a very interesting paper by HORNE[27] This disease is restricted to the olive, and is due to *Bacterium savastanoi* E. F. Smith. It causes knots very similar to the crown gall, but restricted entirely to the branches. It is distributed by means of a slime which oozes from the galls during the rainy weather. Inoculation may occur through natural cracks in the bark.

FAWCETT[28] describes some very interesting citrus galls from Southern California. He believes them to be different from the Jamaica lime and orange knot, which is due to *Sphaeropsis tumefaciens* Hedges.

One of the most interesting cecidia is reported by AMUNDSEN,[29] who found it on wistarias imported from Japan. It occurs on the stems at the base of the buds and in most cases causes the death of the affected buds. Only the pink-flowered plants were infected, and in commenting on this point the author says "whether the fly which laid the eggs discriminated against all the plants which ultimately would produce flowers of other colors and could pick out the pink, or whether the pink varieties were grown in a different locality than the others, could not be ascertained and is still a mystery."

HOUSER[30] describes a gooseberry gall as follows: "The plant is injured by the insect working during the larval stage in the terminal buds of spurs and branches, causing the buds to become abnormal both in size and structure. The bud scales increase greatly in number and size, and lying closely one upon another form a gall somewhat resembling in miniature the pine cone willow galls so commonly encountered upon the tips of willow twigs." Secondary buds are produced and become infected, thus forming a cluster of small cone-shaped galls. The shoots from these galls give a witches' broom effect. The

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[25] ESSIG, E. O., The potato eelworm. Monthly Bull. State Comm. Hort. (Cal.) **1**:26–30. 1911.

[26] CRAWFORD, D. L., A new insect pest. *Op. cit.* **1**:86, 87. 1912.

[27] HORNE, W. T., The olive knot. *Ibid.* **1**:592–600. 1912.

[28] FAWCETT, H. S., Citrus galls. *Ibid.* **1**:937–940. 1912.

[29] AMUNDSEN, E. O., Wistaria gall fly. *Ibid.* **1**:730–733. 1912.

[30] HOUSER, J. S., The gooseberry gall midge or bud deformer. Jour. Econ. Entomol. **5**:180–184.

author also describes the insect, gives its life history, and suggests methods of control.—MEL. T. COOK.

Paleobotanical notes.—Miss HOLDEN[31] has investigated specimens of a conifer from the Trias of New Brunswick which she refers to *Voltzia coburgensis* Schaur. She finds that the foliage of this species is araucarian, the organization of the cone abietineous, and the anatomical structure intermediate between these two groups. This species of *Voltzia*, therefore, represents another early mesozoic form which may be regarded as transition from the Abietineae to the Araucarineae.

Miss BANCROFT[32] has added to the evidence of the uniformity of the mesozoic floras in describing some fossil gymnosperms obtained from the Jurassic of India. The cycadophyte remains are of the *Williamsonia* type, and the vegetative organs show a combination of the characters of Bennettitales and Cycadales.

Miss BANCROFT[33] has described a new stem genus (*Rhexoxylon*) from the later Paleozoic of South Africa. Its general structure suggests relationship with *Medullosa* and *Steloxylon*, and it seems certainly to be an addition to the Medulloseae. A peculiar feature is the character of the inner series of vascular strands, each one consisting of two parts, the outer small and normally oriented, the inner larger and inversely oriented, the two parts being almost in contact. An outer series of strands consists of normally oriented xylem. As contrasted with medullosean stems in general, the wood is compact, the medullary rays are uniseriate, and the pitting of the tracheids is biseriate.

SEWARD and Miss BANCROFT[34] have added to the list of species of Scottish Jurassic plants, describing new species in *Thinnfeldia*, *Brachyphyllum*, *Masculostrobus*, *Conites*, *Strobilites*, and *Cedroxylon*.

SEWARD[35] has published a memoir dealing with two collections of mesozoic plants: the principal one from Afghanistan, made by Mr. H. H. HAYDEN in 1907; the other from Turkistan, made by Mr. GRIESBACH. The composition of the Afghanistan flora is interesting, including the following great groups: Equisetales (several species of *Equisetites*), Filicales (one water fern and several true ferns, including a new genus, *Haydenia*, of Cyatheaceae), Ginkgoales (3 genera), Bennettitales (7 species, among them a new *Williamsonia* and a new *Nilssonia*),

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[31] HOLDEN, RUTH, Some fossil plants from Eastern Canada. Ann. Botany **27**: 243–255. *pls. 22, 23.* 1913.

[32] BANCROFT, NELLIE, On some Indian Jurassic gymnosperms. Trans. Linn. Soc. London II. Bot. **8**:69–86. *pls. 7–9.* 1913.

[33] BANCROFT, NELLIE, *Rhexoxylon africanum*, a new medullosean stem. *Ibid.* 87–103. *pls. 10, 11.* 1913.

[34] SEWARD, A. C., and BANCROFT, N., Jurassic plants from Cromarty and Sutherland, Scotland. Trans. Roy. Soc. Edinburgh **48**:867–888. *pls. 1, 2.* 1913.

[35] SEWARD, A. C., Mesozoic plants from Afghanistan and Afghan-Turkistan. Mem. Geol. Surv. India N.S. **4**: no. 4. pp. 57. *pls. 7.* 1912.

5 species of *Podozamites* (3 of which are new), and Coniferales (5 species, among them a new *Cupressinoxylon*).

SEWARD,[36] in reporting upon a collection of fossil plants from the Wealden of Sussex, describes new species in *Lycopodites*, *Selaginellites*, *Hausmannia* (Dipteridineae), *Pelletieria* (a new genus of Schizaeaceae), *Teilhardia* (a new genus of ferns of uncertain affinity), *Dichopteris*, *Conites* (cones of uncertain affinity). In a general survey of the Wealden floras, the author concludes that "while there is a very close similarity between the Wealden flora of England and the corresponding floras in Eastern and Western North America, the number of cosmopolitan types is smaller than in the case of the Middle Jurassic floras."—**J. M. C.**

Chondriosomes and myelin forms.—The problem of the chondriosomes, or mitochondria, is approached from a new direction in a short article by LÖWSCHIN[37] who happened to notice the formation of myelin forms[38] from lecithin in a microscopic preparation. These myelin forms bore such a remarkable resemblance to chondriosomes that LÖWSCHIN made a careful examination of myelin forms secured from commercial lecithin. The following are some of the more important results: all the forms characteristic of chondriosomes were obtained and their size varied from structures easily seen with a low-power dry objective to those barely visible under the highest powers. In general, the size depends upon the mass of the material, the fineness of its division, and the chemical and physical characteristics of the surrounding medium; while the form is dependent upon the composition of the bodies, their surface tension, and the nature of the surrounding medium. It is to be noted that the elongated forms are found when there is streaming in the surrounding medium. The myelin forms may appear homogeneous or may show a finer structure, and the outer membrane may be liquid or may have more consistency. In many cases a longitudinal splitting, like that described by LEWITSKY for chondriosomes, was observed. The myelin forms arise, develop, and disappear. They may swell and flow together, forming homogeneous threads (*Chondriokonten*), from which are developed granular threads (*Chondriomiten*), which may then break up into single granules (mitochondria), and these again may form into chains. One can observe directly the formation of diplosomes and their division into two granules. The myelin forms, like chondriosomes, may be fixed by chromic acid, osmic acid, or formalin, but are destroyed by acetic acid.

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[36] SEWARD, A. C., Contribution to our knowledge of Wealden floras. Quart. Jour. Geol. Soc. **69**:85–116. *pls. 11–14.* 1913.

[37] LÖWSCHIN, A. M., Myelinformen und Chondriosomen. Ber. Deutsch. Bot. Gesells. **31**:203–209. 1913.

[38] By "myelin forms" is meant the emulsion forms which, under the action of emulsion-producing substances, form upon fatty acids.

While LÖWSCHIN admits that at present he is describing analogies, still he believes that these are too numerous and too striking to be merely accidental.—CHARLES J. CHAMBERLAIN.

Recent work among gymnosperms.—SAXTON[39] has investigated one of the two species of *Actinostrobus*, an endemic Australian genus, and therefore well worth investigation. An outline of the results is as follows. The microsporophyll bears three sporangia and about three months elapse between pollination and fertilization. The archegonia are numerous and deep-seated, "a group of 25–30 being found abutting on the lower end of each pollen tube, which reaches about halfway down the prothallus," the older cells of which are generally 2-nucleate or 4-nucleate. In proembryo-formation, walls are formed when the two free nuclei divide, so that there is a 4-celled proembryo. The completed proembryo, consisting of few cells, fills the egg. Each cell of the proembryo (with perhaps the exception of the two "apical cells") gives rise to a suspensor and an embryo-initial, being as independent in embryo-formation as are the proembryonal cells of *Ephedra*. The chromosome numbers are 8 and 16.

TAKEDA[40] has studied in detail the anatomy of the leaf of *Welwitschia* and concludes that the evidence is all in favor of the Gnetales being gymnosperms, as opposed to the view of LIGNIER and TISON. Even the tracheae, the most striking angiospermous anatomical feature, are in a transition stage, showing incomplete perforations.

TAKEDA[41] has developed a theory of the so-called "transfusion tissue" of gymnosperms. He finds that the "orthodox" transfusion tissue always arises laterally, and is quite independent of centripetal xylem. Therefore, it is not a vestige of the centripetal xylem and is not to be regarded as of phylogenetic significance, its function being "water-storing."—J. M. C.

Gemmae in Radula.—The development of gemmae in two species of *Radula* has been studied by Miss WILLISTON.[42] In *R. flaccida*, a native of tropical America, the gemmae occur on the dorsal margin of the leaves, and formation begins by the enlargement of a single cell around which a transparent gelatinous substance is secreted. A periclinal wall divides the gemma initial into a stalk cell which undergoes no further division, and an outer or mother cell which is divided by an anticlinal wall. The next division gives a quadrant, the two outer cells of which immediately function as apical cells with two cutting faces. The two inner cells of the quadrant do not produce apical cells.

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[39] SAXTON, W. T., Contributions to the life history of *Actinostrobus pyramidalis* Miq. Ann. Botany **27**:321–345. *pls. 25–28.* 1913.

[40] TAKEDA, H., Some points in the anatomy of the leaf of *Welwitschia mirabilis*. Ann. Botany **27**:347–357. *pl. 29.* 1913.

[41] TAKEDA, H., A theory of "transfusion tissue." Ann. Botany **27**:359–363. 1913.

[42] WILLISTON, RUTH, Bull. Torr. Bot. Club **39**:329–339. *figs. 37.* 1912.

The gemmae at maturity measure 0.5 mm. in diameter. The adult leaf measures 0.8 mm. in diameter. In *R. protensa*, a native of New Guinea and adjacent regions, the initial becomes covered with a greater quantity of gelatinous material than in *R. flaccida*. As the gemma increases in size it finally bursts through the gelatinous covering, which then clings to the base like a collar.

The gemmae of *Radula* are arranged in two groups according to complexity. In the first group the gemmae occur on margins of leaves, are irregular in outline when mature, and may be more than one cell thick; in the second they occur on the margin and surface of leaves, are regular in development and symmetrical in form, and are only one cell thick. *R. flaccida* and *R. protensa* belong to the second class.—W. J. G. LAND.

Cytology of Hymenomycetes.—LEVINE,[43] working in Harper's laboratory, has investigated the carpophores of 24 species of *Boletus* and of several species of *Polyporus*, has had in cultures the mycelia of various Hymenomycetes, and has secured some spore germination (none of the spores of *Boletus* germinated), so that his observations of the nuclear phenomena are somewhat extensive. The germinating spores of *Pholiota praecox* produce multinucleate germ tubes; in cultures 48 hours old the cells of the mycelium are multinucleate; but in cultures 3 days old, both uninucleate and binucleate cells are found. The mycelial cells of many species are binucleate, with clamp connections, etc. In the mature stipe of *Boletus granulatus* all the cells are multinucleate; while those of the ring, of the flesh and trama, and of the subhymenium are binucleate. At the end of the second division of the fusion nucleus in the basidium, the centrosomes become attached to the walls of the basidium and the 4 daughter nuclei remain connected with them by fibrillar strands. The centrosomes determine the points of origin of the 4 sterigmata and are carried up with the growth of the sterigmata and into the spores, pulling the nuclei into the spores. All the spores studied were uninucleate at first. The conclusion is that an alternation of generations "comparable to that in the Uredineae" is also present in these forms. "The sporophyte begins at some indefinite point in the mycelium and extends through the development of the carpophore."—J. M. C.

Aluminium salts.—FLURI[44] has claimed that aluminium salts render certain plant cells incapable of being plasmolyzed by ordinary plasmolytic agents by rendering the protoplasm highly permeable to these reagents. SZÜCS[45] finds that the protoplasm is rendered less permeable to many agents by aluminium salts,

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[43] LEVINE, MICHAEL, Studies in the cytology of the Hymenomycetes, especially the Boleti. Bull. Torr. Bot. Club **40**:137–181. *pls. 4–8.* 1913.

[44] BOT. GAZ. **47**:252. 1909.

[45] SZÜCS, JOSEPH, Über einige characterische Wirkung des Aluminiumions auf das Protoplasma. Jahrb. Wiss. Bot. **52**:269–332. 1913.

and that the incapacity for plasmolysis is due to a hardening of the protoplasm. The main evidence for the hardening of the protoplasm is the failure of chloroplasts to be displaced by a centrifugal force of about 1000 gravities, which ordinarily displaces them readily. This process of hardening is reversed (physicochemically and physiologically) by transferring the cells to aluminium-free solutions. The slow rate of reversing leads the author to postulate the tying up of the aluminium in an indiffusible form. It is only in moderate concentrations that aluminium salts produce the effect; in higher concentrations the protoplasm does not harden. Anthocyanin cells cannot be thus hardened, probably due to high sugar content. The author relates this to the fact that non-electrolytes hinder the precipitation of proteins by ions. He also thinks that aluminium salts are specific only in that their low toxicity enables them to bring about such a fundamental physical change in the protoplasm without killing it. While most salts produce such a change, it is not physiologically reversible.—WILLIAM CROCKER.

Individuality of the chromosome.—A four-years' study of the vegetative and reproductive nuclei of *Carex aquatilis* has brought STOUT[46] to the conclusion that the chromosome is an individual organ, maintaining its identity through successive cell generations. He finds that even in resting nuclei the chromosomes are visible as definite bodies which can be counted, and that these chromosomes can be traced through all the stages of vegetative and reduction divisions, except synapsis. Spiremes are formed in both vegetative and reduction divisions, but even in the spirem the individual chromosomes are distinguishable. In the vegetative spirem there is no evidence of any splitting, the longitudinal splitting of the chromosome appearing only after the chromosomes have become arranged in the nuclear plate. In the metaphase of the heterotypic mitosis the chromosomes form a more or less obvious double spirem. The heterotypic mitosis separates whole chromosomes which have been previously paired. Although the study would have been more satisfactory if the stages in synapsis had been more complete, the results form an important addition to the already strong evidence that the chromosome is an individual organ. *Carex aquatilis*, like *Carex acuta* described by JUEL, forms only one pollen grain from a pollen mother cell, the other three aborting at an early stage.—CHARLES J. CHAMBERLAIN.

Seedling anatomy.—HILL and DEFRAINE[47] have worked long enough upon seedling anatomy to have come to some very interesting conclusions. Citing numerous facts that have been used in phylogenetic conclusions, they state that they "see no necessity for preserving seedling anatomy from the fate already meted out to other structural features which were at one time considered as

[46] STOUT, A. B., The individuality of the chromosomes and their serial arrangement in *Carex aquatilis*. Archiv. Zellforschung **9**: 114–140. *pls. 11, 12.* 1912.

[47] HILL, T. G., and DEFRAINE, E., A consideration of the facts relating to the structure of seedlings. Ann. Botany **27**: 257–272. 1913.

indicators of phylogeny." "In fact, until more knowledge is obtained with regard to the interrelationship of plant members and the influence of environment—in a word, the influence of physiological necessity on morphological expression—we cannot determine with any degree of certainty the precise value of many anatomical characters." The same disposition is made of the size and number of vascular bundles in connection with the transition phenomena. "Sufficient has been said to show the enormous importance of physiology in questions relating to vascular tissues; for our own part we are strongly of the opinion that no real further advance in our knowledge of morphology, more especially of the higher plants, is possible without an adequate investigation of the physiology of the members concerned."—J. M. C.

Inheritance of quantitative characters.—EMERSON and EAST[48] have discussed this subject rather fully and have presented data bearing upon it secured from experiments with maize. Inheritance was studied in number of rows per ear, length of ear, diameter of ear, weight of seeds, breadth of seeds, and height of plants. The general conclusion is stated compactly as follows: "The results secured in the experiments with maize were what might well be expected if quantitative differences were due to numerous factors inherited in a strictly Mendelian manner. It is quite likely that genetic correlations occur between factors for distinct quantitative characters. These and the physiological correlations so frequently noted make the results more difficult of interpretation, but do not throw them out of the realm of Mendelian phenomena. Physiological correlation is a phenomenon of development, not of inheritance, and as such has less interest for students of genetics than for experimental morphologists. Even in practical plant breeding, correlations of this sort are of importance mainly on account of the physiological or morphological limits that they set to the perfect development of particular combinations of characters."—J. M. C.

The individuality of the plastid.—In a preliminary paper[49] published two years ago, Sapĕhin found plastids even in sporogenous tissue, both in monoplastic types, like *Anthoceros*, *Isoetes*, and *Selaginella*, and in polyplastic forms, like the majority of plants. In a second preliminary account[50] he deals principally with *Lycopodium*, which he finds to belong to the monoplastic type; and with *Funaria*, which belongs to the polyplastic type. In the antheridium of *Funaria*, which starts as a polyplastic organ, cell division is not accompanied by any division of the plastid, and consequently the spermatogenous cells soon become

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[48] EMERSON, R. A., and EAST, E. M., The inheritance of quantitative characters in maize. Agric. Exper. Station, Univ. Neb., Research Bull. 2. pp. 120. *figs.* 21. 1913.

[49] SAPĔHIN, A. A., Über das Verhalten der Plastiden in sporogenen Gewebe. Ber. Deutsch. Bot. Gesells. **29**:491–496. *figs.* 5. 1911.

[50] ———, Untersuchung über die Individualität der Plastide. Ber. Deutsch. Bot. Gesells. **31**:14–16. 1913.

monoplastic; each sperm receives one plastid. SAPĚHIN claims that the bodies described by ALLEN, in the spermatogenesis of *Polytrichum*, as blepharoplasts, are nothing but plastids. Since SAPĚHIN's account is not illustrated, one must feel rather skeptical in regard to his interpretation of ALLEN's work. —CHARLES J. CHAMBERLAIN.

Botrychium Lunaria.—LANG[51] has published the first of a series of papers on the vascular anatomy of the Ophioglossaceae. From examination of *Botrychium Lunaria* he concludes that the internal endodermis is a new formation without morphological significance. The occurrence of occasional tracheids in the pith, especially in injured plants, is accepted as conclusive evidence of the stelar nature of the pith. These scattered tracheids are primary in their origin and by reason of their position are identified as centripetal xylem; hence this species of *Botrychium* has mesarch stem structure.

Axillary buds are of constant occurrence in this species, as in *Helminthostachys*. These develop only in case of injury to the terminal bud. The vascular supply of the branch arises from the trace of the leaf immediately below by the development of adaxial xylem; occasionally the xylem of the branch may be connected directly with that of the main stem.—L. C. PETRY.

Lichens of the Galapagos Islands.—STEWART[52] has reported on the lichens collected by the expedition of the California Academy of Sciences to the Galapagos Islands in 1905–1906. These islands have long been of great biological interest, so that any collection from them promises to be worth noting. The list numbers 47 species, 7 of which are undetermined. The genera represented are as follows: *Alectoria* (1 sp.), *Arthonia* (4 spp.), *Buellia* (2 spp.), *Chiodecton* (1 sp.), *Cladonia* (6 spp.), *Coenogonium* (1 sp.), *Lecanora* (3 spp.), *Lecidea* (1 sp.), *Pannaria* (1 sp.), *Parmelia* (4 spp.), *Pertusaria* (2 spp.), *Physcia* (2 spp.), *Placodium* (1 sp.), *Pyrenula* (1 sp.), *Ramalina* (5 spp.), *Rinodina* (1 sp.), *Roccella* (2 spp.), *Sticta* (2 spp.), *Teloschistes* (1 sp.), *Usnea* (4 spp.), *Verrucaria* (1 sp.).—J. M. C.

Plants of northwestern Canada.—STANDLEY[53] has determined the plants collected by an expedition to the Mount Robson region of eastern British Columbia and western Alberta in 1911, under the auspices of the Alpine Club, whose headquarters are at Banff, Alberta. The collection includes about 200 numbers, all of which are angiosperms except 5 mosses, 6 pteridophytes, and 3 gymnosperms. A new species was discovered in each of the following genera: *Carex*, *Vagnera*, *Artemisia*, *Aster*, *Gaillardia*.—J. M. C.

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[51] LANG, WILLIAM H., Studies in the morphology and anatomy of the Ophioglossaceae. I. On the branching of *Botrychium Lunaria*, with notes on the anatomy of young and old rhizomes. Ann. Botany **27**: 203–242. *figs. 1–14. pls. 20–21.* 1913.

[52] STEWART, ALBAN, Notes on the lichens of the Galapagos Islands. Proc. Calif. Acad. Sci. IV. **1**: 431–446. 1912.

[53] STANDLEY, PAUL C., Plants of the Alpine Club expedition to the Mount Robson region. Canadian Alpine Journal, special number, pp. 76–97. 1912.